THE ANALYST

Safety in Numbers

It is now about 30 years since analysts involved in various aspects of food and pharmaceutical technology took what some of their colleagues appeared to regard as a bold, not to say a reckless, step. The current methods of biological assay, into which they had been more or less pitchforked because there were no other victims handy and—after all—measuring a vitamin in a rat was, although admittedly a *pis aller*, only an alternative to weighing it on a balance, soon produced a state of profound disquiet in their analytical consciences. Did the results they were getting really mean anything? Could they be made to mean anything? Above all, how could they find out whether they did or could mean anything?

Some one—and it would probably be neither rewarding nor helpful to try and find out who it was—noted the incursion of statistical concepts and methods of design in the somewhat remote, at any rate to most analysts, discipline of pharmacology. It occurred to him that the scientific principles applicable to measuring effects on animals might be what he was looking for in his efforts to assess amounts of, say, the antirachitic vitamin, at that time recognised but not isolated. He, or she, began to talk meaningly of variances and correlation coefficients.

The thing caught on. It was not long before a dozen or more laboratories began to apply to their results the critical probe afforded by the new, or newly realised, technique. Revision of experimental design to meet the stringent requirements of significance tests became at one and the same time the curse and the blessing of those engaged in what was coming to be known as bio-assay. The method of least squares supplanted the ruler, and the mean deviation gave way to the standard error.

It is not necessary to dwell at any length on the importance of this newly developed branch of "analytical chemistry" or, as the first Chairman of our own Biological Methods Group, in his introductory address, urged us rather to call it, "chemical analysis." The existence of the Group bears witness to the Society's view that here were scientific procedures worthy of detailed discussion by specialists, in the same way as physical methods and micro-analysis. The many stimulating meetings organised by the Group are a sufficient justification of the Society's attitude. Yet there is another less obvious benefit to be put to the credit of the statistical developments of bio-assay, in general, and perhaps to some extent also to the Biological Methods Group in particular.

For the analyst turned bio-assayist, particularly if he occupied an industrial post, did not have his being in even a partial vacuum. He was in daily contact with other chemists, engaged in production, analysis and research. His new approach in due course had its effect on the others' outlooks. Probability levels and fiducial limits eventually came to mean something to them also. It was inevitable for them, for some of them, anyhow, to ask themselves about their colleague's new technique whether there might not be, as the saying is, "something in it for us." Nor, it must be allowed, was his influence operating entirely unaided.

A tiny minority of technologists who were involved in such things as the sampling of coal and the quality of electric-light bulbs had already seen the light of the early statistical dawn. Some of its rays must also have reached the chemist. Whatever the relative strengths of these two influences, it was not long before the growing demand for knowledge of how to apply statistical concepts and methods to control and development work began to be met by lectures, monographs and large books of imposing proportions. Since the war there have been several published in English, on both sides of the Atlantic, in which the theory and practice of statistical design and analysis have been expounded for the specific

assistance of those engaged in the development and control of industrial processes. The non-biological analyst is among the beneficiaries, and few investigations into quantitative analysis, especially the increasing number conducted on a collaborative basis, are to-day planned without due consideration of statistical aspects from the outset.

It is often said, perhaps a little by way of a trite generalisation, that advances in any one field of chemistry can help those working in other fields. In the mid-twentieth century the use of statistical weapons over the whole of applied chemistry, from bio-assay to plant design, is surely an outstanding example of how a benefit to one may become a benefit to all.

A. L. BACHARACH

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society, organised by the Microchemistry Group, was held at 7.15 p.m. on Friday, January 27th, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. K. A. Williams, F.R.I.C., A.Inst.P., M.Inst.Pet.

The following papers were presented and discussed: "Microchemical Methods in the Art Gallery and Museum," by A. E. Werner, M.A., M.Sc., D.Phil., A.R.I.C.; "The Ring-oven Technique and its Application in Archaeology," by H. Weisz, Dr.techn.Dipl.-Ing.

NEW MEMBERS

ORDINARY MEMBERS

Frederick Culkin, B.Sc. (Lond.), A.R.I.C.; Albert Arthur Henly, B.Sc., Ph.D. (Lond.), F.R.I.C.; Douglas William James, B.Sc. (Lond.), A.R.C.S.; Roger Millward; Derek Ivor Stansell, B.Sc. (Lond.), A.R.I.C.; Catherine Hannah Tinker, B.Sc., Ph.D. (Lond.), A.R.I.C.

JUNIOR MEMBERS

Alan William Archer, A.R.I.C.; Alan Herbert Helman, A.R.I.C.

SCOTTISH SECTION

THE Twenty-first Annual General Meeting of the Section was held at 5 p.m. on Friday, January 20th, 1956, at the Rhul Restaurant, 123 Sauchiehall Street, Glasgow. The President, Dr. K. A. Williams, F.R.I.C., A.Inst.P., M.Inst.Pet., the Hon. Assistant Secretary, Dr. R. E. Stuckey, F.R.I.C., F.P.S., and 9 founder members were among the 32 present. The Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., presided. The following office bearers were elected for the forthcoming year:—*Chairman*—Dr. F. J. Elliot. *Vice-Chairman*—Dr. Magnus Pyke. *Hon. Secretary and Treasurer*—Mr. J. A. Eggleston, Boots Pure Drug Co. Ltd., Airdrie Works, Airdrie, Lanarkshire. *Members of Committee*—Messrs. J. Brooks, J. W. Gray, J. B. Headridge, R. L. Mitchell, R. S. Watson and W. Wilson. Messrs. J. Andrews and J. McL. Malcolm were appointed as Hon. Auditors.

On behalf of Council and the whole Society, Dr. Williams congratulated the Section on completing its twenty-first year.

The Annual General Meeting was followed at 7.30 p.m. by a film evening, at which the following films were shown: "The Technique of Sampling," "Gravimetric Analysis" Parts I–IV, "Care of Laboratory Glass-ware," "Fractional Distillation (Micro)" and "Principles of Chromatography" (colour).

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, January 11th, 1956, in the Main Chemistry Theatre, Department of Chemistry, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

The following papers were presented and discussed: "Gas Chromatography," by J. C. Tatlow, Ph.D., D.Sc., A.R.I.C.; "Ionophoresis" by A. B. Foster, B.Sc., Ph.D.

THE First Annual General Meeting of the Section was held at 7 p.m. on Tuesday, January 24th, 1956, in the Sale Room, Regent House, St. Philip's Place, Birmingham. The Chairman

of the Section, Mr. J. R. Leech, J.P., presided. The following appointments were made for the ensuing year:—*Chairman*—Mr. J. R. Leech. *Vice-Chairman*—Dr. R. Belcher. *Hon. Secretary*—Mr. G. W. Cherry, 48 George Frederick Road, Sutton Coldfield, Warwicks. *Hon. Treasurer*—Mr. F. C. J. Poulton. *Members of Committee*—Mr. R. Adkins, Dr. Bella B. Bauminger, Messrs. H. J. G. Challis, F. P. Everett, S. H. Jenkins, C. A. Johnson, H. C. Smith, W. H. Stephenson and T. S. West. Mr. H. J. Alcock and Miss M. E. Tunnicliffe were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section at which a paper on "Ultra-micro Methods for the Analysis of Organic Compounds" was given by T. S. West, B.Sc., Ph.D., A.R.I.C.

An Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, February 7th, 1956, in the Mason Lecture Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

The following papers were presented and discussed: "The Analytical Chemistry of Germanium" by H. J. Cluley, M.Sc., F.R.I.C.; "The Analytical Chemistry of Gallium," by G. W. C. Milner, M.Sc., F.R.I.C., A.Inst.P.

MICROCHEMISTRY GROUP

The Twelfth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, January 27th, 1956, in the meeting room of the Chemical Society, Burlington House, Piccadilly, London, W.1. In the absence of the Chairman of the Group, the Vice-Chairman, Mr. D. F. Phillips, A.R.I.C., presided. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. G. F. Hodsman. *Vice-Chairman*—Mr. D. F. Phillips. *Hon. Secretary*—Mr. D. W. Wilson, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Messrs. P. R. W. Baker, R. Belcher, R. A. Chalmers, F. Holmes, M. A. Fill and A. M. Ward. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Hon. Auditors.

The Annual General Meeting was followed, at 7.15 p.m., by an Ordinary Meeting of the Society, organised by the Microchemistry Group, at which the President, Dr. K. A. Williams, F.R.I.C., A.Inst.P., M.Inst.Pet., presided.

SUMMARIES OF PAPERS PRESENTED AT MEETINGS OF THE SOCIETY

The following are summaries of the papers presented at the Ordinary Meeting of the Society organised by the Biological Methods Group on Wednesday, November 2nd, 1955, in London. A first report appeared in *The Analyst*, 1955, **80**, 781.

The subject of the meeting was "The Evaluation of Anti-fungals," and the following papers were presented and discussed: "Laboratory Evaluation of Drugs for Clinical Trial Against Dermatomycoses," by H. O. J. Collier, B.A., Ph.D., M.I.Biol., and G. K. A. Smith, A.I.M.L.T.; "Some Factors in the Planning of Fungitoxicity Experiments in the Laboratory," by R. J. W. Byrde, B.Sc., Ph.D., and G. M. Clarke, M.A., Dip.Stat.; "Cattle Ringworm: Problems in the Evaluation of Treatment," by K. C. Sellers, Ph.D., B.Sc., D.V.S.M., M.R.C.V.S.

Laboratory Evaluation of Drugs for Clinical Trial Against Dermatomycoses

Mr. Smith said that in the evaluation of potential remedies against dermatophytes, skin infections of laboratory animals seldom appeared to have been used. Nevertheless, for this purpose both natural and experimental infections could be considered. Natural fungal infections occurred in laboratory mice, their existence being sometimes detected by the appearance of ringworm among laboratory staff. In more severe epidemics among mice, such as those described by B. H. Booth (*Arch. Dermat. Syph., N.Y.*, 1952, **66**, 65), skin lesions or loss of hair were obvious. Such natural infections of mice might be adaptable for chemotherapeutic trial; but adequate methods of maintenance and control of the infection were first required.

Experimental infections of fungi could be obtained in guinea-pigs and rabbits by rubbing fungal cultures into the shaved and scarified skin. E. D. De Lamater and R. W. Benham (*J. Invest. Dermat.*, 1938, **1**, 451) described the production of such infections and summarised the earlier literature. Within 4 to 6 days of inoculation, virulent strains of *Trichophyton* caused skin lesions that were active for about a fortnight

and then healed spontaneously. This type of infection offered opportunities for chemotherapeutic experiments; but at present the vast majority of tests reported in the literature were of the *in vitro* type.

Without *in vivo* therapeutic methods, evaluation of potential remedies rested on a variety of measurements, including those of *in vitro* antifungal activity and of systemic and skin toxicity. For *in vitro* tests, plate methods had been used, for example by H. I. Chinn, R. B. Mitchell and A. C. Arnold (*J. Invest. Dermat.*, 1953, **20**, 177); but methods of dilution in liquid media appeared to be more usual and to have certain advantages. They could, for example, be readily adapted to testing antagonism by materials such as hair and to exploring fungicidal activity. Studies of fungicidal action could not be relied upon unless a potent antagonist of the drug under investigation was incorporated in the medium of sub-cultures. In the bisquaternary ammonium series examined by the present authors (H. O. J. Collier, M. D. Potter, E. P. Taylor and G. K. A. Smith, *Brit. J. Pharmacol.*, 1955, **10**, 343), prolongation of exposure to drug increased fungicidal and reduced fungistatic effects.

In addition to studies of systemic toxicity, the reactions of skin to intradermal injections and repeated application of drugs should be investigated. For both these purposes, the shaved skins of rabbits and guinea-pigs could be used. Hairless mice were also being studied as a means of assessing the toxicity of drugs applied repeatedly to skin.

Knowledge of the penetration of antifungal substances into keratin and hair follicles might also contribute to their evaluation; but at present, except with coloured or fluorescent substances, penetration was difficult to assess experimentally. It was possible that radioactive methods might later be applicable. A. J. E. Barlow and F. W. Chattaway (*J. Invest. Dermat.*, 1955, **24**, 65) have shown that treatment of hairs with keratolytic agents rendered them more susceptible to invasion by dermatophytes. While this observation threw doubt on the therapeutic value of keratolytic agents in antifungal preparations, it suggested a method of enhancing virulence of fungi in experimental animals by the application of keratolytic agents before the inoculation of the skin.

Some Factors in the Planning of Fungitoxicity Experiments in the Laboratory

Dr. Byrde said that an antifungal assay system might be regarded, very simply, as consisting of three components—fungus, medium and toxicant—each of which would contribute to the over-all variability of the results.

The fungus inoculum should be obtained by standard methods; methods for spore suspensions had been studied in detail by S. E. A. McCallan and F. Wilcoxon (*Contr. Boyce Thompson Inst.*, 1939, **11**, 5).

In the medium, controlled hydrogen-ion concentration was important, particularly in tests of weak electrolytes. In general, such compounds were most active in the undissociated form, owing to greater ease of penetration of the lipophilic cell membrane. For valid comparisons of inherent toxicity, they should be tested at a pH level at which they were predominantly undissociated. This could be achieved by buffering the medium 2 pH units below pK (for weak acids) or 2 pH units above pK (for weak bases). If, however, it was desired to apply the results of a test to a specific *in vivo* system, it was preferable to work at the pH of that system, as did E. W. Simon and G. E. Blackman ("Report of Third Symposium of the Society of Experimental Biology, 1949," p. 253).

In general, the medium should be the simplest that would permit normal growth of the fungus, and, if possible, should consist of specific compounds, *e.g.*, asparagin, rather than biological extracts.

The performance of a fungicide may be profoundly modified by its physical form, as described by G. L. McNew and R. P. Burchfield (*Contr. Boyce Thompson Inst.*, 1950, **16**, 163). That the presence of a mutual solvent may enhance fungistatic activity was shown by R. J. W. Byrde and D. Woodcock (*Ann. Appl. Biol.*, 1953, **40**, 675).

The assay of volatile fungicides presented a special problem, owing to the decreasing concentration in the medium, and results from such experiments should be interpreted with caution.

MR. CLARKE said that in laboratory comparisons of the effectiveness of fungicides, carried out in incubators, sources of variation in growth of fungal material were common characteristics of the incubators themselves independently of the applied fungicide treatments: vertical trends were often present, growth being better at the top, and horizontal trends might also exist. It was also useful to examine whether the replicates of a particular treatment showed any trend due to the order of pouring out of the applied fungicide from its stock preparation. Work along these lines has been described by G. V. Coles, J. T. Martin, and R. J. W. Byrde (*Ann. Rep. Long Ashton Res. Sta.*, 1954) and by G. M. Clarke (*Appl. Statistics*, 1955, **4**, 199). Where there was not scope for eliminating all these effects, a randomised block design might be used, each vertical layer in the incubator forming one complete block. In order to compare the results of several experiments, a standard substance must be included in each run, and would vary slightly in its effect; but the other compounds in each trial could be compared with this standard, and hence indirectly with compounds in other trials. Some fungi had much higher natural variabilities than others, and so if a choice existed, a less variable organism was chosen to make experimental results as precise as possible. Moreover, it was not possible to compare two fungi of different variances by means of a single analysis of variance, nor therefore always directly to compare the effect of a fungicide on two different fungi.

CATTLE RINGWORM: PROBLEMS IN THE EVALUATION OF TREATMENT

DR. SELLERS said recent work by G. C. Ainsworth and P. K. C. Austwick (*Vet. Rec.*, 1955, **67**, 99) and by K. C. Sellers, C. J. La Touche and W. B. V. Sinclair (in preparation) had shown that *Trichophyton verrucosum* Bodin var. *discoides* Georg was the most common cause of cattle ringworm in this country. In Yorkshire the infection was found on a high proportion of farms, more commonly in calves in their first year of life and during the winter. Humans contracted the infection, but no accurate estimate of the incidence in this species had been made. As a preliminary to work on the control and treatment of cattle ringworm in the field, experimental observations had been carried out at the Veterinary Investigation Laboratory, Ministry of Agriculture, Leeds, and the Skin Department, Leeds General Infirmary. The results showed that healthy calves could be infected experimentally with material from natural cases of ringworm or with culture suspensions. Histological examinations of experimentally induced and naturally occurring lesion were made at various stages during the course of the disease. These surveys and experimental studies had shown that, although satisfactory trials might be performed in the artificially infected calf, the final and accurate assessments of an antifungal under field conditions presented many problems. The remedy, for use under farm conditions, must be easy to apply and persistent in action. It must also penetrate through the crust to the depth of the infected hair and follicle, and must not damage the hide nor be toxic to the calf. Other factors such as the age of the lesion and the state of nutrition of the animal may influence an animal's response to treatment. Consideration should also be given to the use of fungicidal or fungistatic paint or sprays for dressing farm buildings where reservoirs of infection might persist. Nevertheless, cattle ringworm lent itself to well planned clinical trials; not only was it widespread, but ethical and cosmetic considerations did not bulk so largely as in man. Also, it was possible that the results so gained would be applicable to the ringworms of other species.

Obituary

OSMAN JONES

OSMAN JONES died in hospital at Oxford on June 22nd, 1955, after a very brief illness. It came as a great shock to his friends and colleagues as, feeling the strain of overwork, he had entered the hospital only a few days previously for observation.

Born in 1886, Osman Jones was articled to J. Kear-Colwell, Public Analyst for St. Pancras, Holborn, Bedford and Luton in 1903. During this period he attended the City and Guilds of London Institute when Sylvanus Thompson was the Principal and Meldola the Professor in the Department of Technical Chemistry, and gained the Diploma in this Department in 1908. In 1912 he was appointed Chief Chemist to the English Margarine Works, Broad Green, Liverpool, where he remained until 1918. He was then appointed Chief Chemist to Messrs. C. & T. Harris (Calne) Ltd. and began his long association with the meat industry, which was in effect his life's work. During this period he was responsible for inauguration of the By-Products Factory and also the Water Softening Plant, giving to both projects his usual unbounding enthusiasm and energy. He remained at Calne until 1946, when he was appointed Scientific Advisor to Messrs. Lovell and Christmas, and was responsible for organising the St. John's Laboratory, of which he was in control until his death.

During his long career Osman Jones was actively connected with many professional and learned societies. Elected a fellow of the Royal Institute of Chemistry in 1922, he served on the Council from 1943 to 1946 and was Streatfeild Memorial Lecturer in 1944, choosing as his subject "Modern Methods of Food Preservation." It will be remembered that Streatfeild was a Lecturer at the Finsbury Technical College during his period as a student. He was a member also of the other two chartered chemical societies, as well as the Society for Analytical Chemistry, the Society for General Microbiology, the Society for Applied Bacteriology, the American Chemical Society, the Institute of Refrigeration and the Institute of Meat. Active membership of these many bodies does not complete the story of Osman Jones's work for the advancement of Food Science nor his interest in the social aspects of his life's work. A staunch member of the Chemical Club, he was for many years a member of its Executive Committee. In 1937 he was appointed Chairman of the Meat and Fish Products Panel of the British Food Manufacturers Research Association. He was an active member of the Meat Extract Sub-Committee of the Analytical Methods Committee and had accepted membership of the newly formed Meat Products Sub-Committee.

Osman Jones will long be remembered as a leading British expert on the technology of meat and bacon, a reputation that he also held in Continental countries, especially Holland, which he visited frequently in the course of his duties. His book "Canning Practice and Control," first published in 1937 and since issued in two revised editions, is as well known to food technologists as are the many articles that he wrote for the technical press, often on very controversial subjects on which he held strong and, not infrequently, individualistic views.

Little need be written on his skill as a food chemist nor on the broadness and originality of his outlook, as these are only too well known by his colleagues and friends. Equally well known was his readiness and eagerness to be of assistance even when pressure of a very full life made this seem well nigh impossible.

Osman Jones's home life was of the happiest. His chief hobby was gardening, especially rose growing; he had a wonderful collection. He is survived by his wife and three married daughters, to whom and to his grandchildren he was devoted.

H. G. REES

The Separation of Zinc from Some Other Elements by Means of Anion Exchange and Solvent Extraction, and its Titrimetric Determination with Disodium Ethylenediaminetetra-acetate

By JOHN A. HUNTER* and CHRISTINA C. MILLER

(*Presented at the meeting of the Scottish Section on Thursday, April 28th, 1955*)

Previous work on the separation of zinc from other elements has been extended to include cadmium, gallium and indium. Zinc is adsorbed from 2 *N* hydrochloric acid solution by the anion-exchange resin, Amberlite IRA-400(Cl). Cadmium is then fixed on the resin by means of hydriodic acid and zinc is eluted with water and 0·25 *N* nitric acid. Much tin, some molybdenum, a little indium, lead, platinum, titanium and tungsten (as phosphotungstate), and a very little antimony, copper, gallium, iron^{III}, manganese, thallium, thorium, uranium and zirconium may accompany zinc. Pre-treatment of the resin with methylarsonic acid, and addition of some to the sample solution before transfer to the resin, greatly reduce the amount of tin accompanying zinc.

Quantities of zinc ranging from 0·1 to 5 mg are titrated with disodium ethylenediaminetetra-acetate solution, Solochrome black being used as indicator in a spectrophotometric determination of the end-point. The small amounts of all the above-named elements accompanying zinc, except gallium, indium, titanium and uranium, can be tolerated. These four elements, together with lead and tin, can be eliminated by chloroform extraction of zinc as zinc pyridine thiocyanate from the eluates collected from the resin. The zinc, together with copper, manganese and iron^{II}, is withdrawn from the chloroform into an ammoniacal solution and titrated as usual.

For the determination of 5-mg and 0·5-mg amounts of zinc in mixtures in which the total cation content may be 100 mg, the results show no significant systematic error. The standard deviations are 15 and 8 μg, respectively. Results are given for a few alloys and a glass.

Recently,[1] we showed that the adsorption of zinc by a strong-base anion-exchange resin, from a solution 2 *N* in hydrochloric acid,[2] could be utilised for separating it from aluminium, beryllium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, thorium, titanium, uranium and zirconium, which show no, or only slight, affinity for the resin under the specified conditions. Antimony, bismuth, cadmium, tin and a little indium and lead accompanied zinc on the resin and, when the zinc was eluted by means of water and dilute nitric acid, all the cadmium and indium, a considerable proportion of the tin, some antimony, a little bismuth and lead, and traces of copper and iron, and perhaps of certain other elements, were simultaneously eluted. In experiments with 5 to 50 mg of zinc in synthetic solutions, or in alloys containing a maximum of 100 mg of the above-mentioned elements, excluding cadmium and indium, zinc, after separation by anion exchange, was determined gravimetrically by means of 8-hydroxyquinoline, under conditions in which all potential interference, save from a trace of copper, was suppressed. In a few experiments involving micro-techniques, the quantitative separation of 0·5 mg of zinc from 100 mg of aluminium or magnesium was achieved. Recently, Rush and Yoe[3] have used a similar anion-exchange process for separating 0·02 to 1 per cent. of zinc in a few materials before its colorimetric determination.

The general procedure of our earlier experiments has now been extended to cover the quantitative separation of zinc and cadmium, and also the separation and determination of 0·5-mg amounts of zinc in association with many other elements besides aluminium and magnesium. For the determination of 5 to 50 mg of zinc, initially associated with cadmium

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and with other elements whose behaviour might be influenced by the introduction of an additional step in the anion-exchange separation, the gravimetric method with 8-hydroxyquinoline has, for convenience, been retained. The use of the disodium salt of ethylenediaminetetra-acetic acid for the titrimetric determination of 0·5-mg to 5-mg quantities of zinc has been comprehensively studied. Adequate accuracy and precision have been obtained by determining end-points spectrophotometrically, with Solochrome black as indicator.

Experimental

Separation of 5 to 50 mg of zinc from cadmium by means of anion exchange—

The previously described method for the adsorption of zinc by the chloride form of the strong-base anion-exchange resin, Amberlite IRA-400, was equally applicable to cadmium. The simplicity and effectiveness of the separation of zinc from many important elements was such that a means was sought of treating the resin on which zinc and cadmium were held, so as to prevent their simultaneous elution, and thus permit the ready determination of zinc. Concentrated hydrochloric acid, fluorides, iodides and other reagents were tested for this purpose and hydriodic acid was found to be the most suitable. Recently, Baggott and Willcocks[4] described a procedure for separating a small amount of zinc from cadmium metal, which depended on the formation of an anionic complex of iodide and cadmium, but not of zinc, in a solution containing sulphates, which was then passed through an appropriately prepared column of the strong-base anion-exchange resin, De-Acidite FF. The cadmium was adsorbed and the zinc passed through. Their method would be unsuitable for solutions containing the large assortment of elements under consideration in this paper.

After the 2 *N* hydrochloric acid solution containing the various elements was transferred to the column and washing with 2 *N* hydrochloric acid was completed, 5 ml of 2 *N* hydriodic acid were added to the column to give complete retention of 100 mg of cadmium. If, instead, 2 ml were used, 0·5 mg of cadmium was subsequently found with the zinc. The effluent was collected and the column was further treated with 15 ml of water and 40 or 60 ml of 0·25 *N* nitric acid; 60 ml led to the recovery of slightly more zinc. In the combined effluents zinc was determined gravimetrically by means of 8-hydroxyquinoline. In order to avoid possible interaction of liberated iodine with the latter, and resultant interference with the determination of zinc, the effluents were treated with a small excess of nitric acid, and cautiously evaporated to break down iodides and expel iodine, before the precipitation of zinc 8-hydroxyquinolinate was effected as previously described.

Of the other elements that were hitherto found to be mainly or completely adsorbed by the resin from 2 *N* hydrochloric acid solution and partly eluted by water and nitric acid, 100 mg of bismuth and 50 of antimony^{III} were now found to yield none to the eluate and 50 mg of antimony^{V} yielded 500 μg. The amount of tin escaping from 100 mg increased from 20 to 30 mg and 1 mg of lead reached the eluate. The behaviour of some other elements, believed not to be significantly adsorbed by the resin, was more closely examined. It was found that 100 mg of chromium^{III}, manganese^{II}, thorium, uranium^{VI} and zirconium yielded none, 20, 10, 100 and 100 μg, respectively, to the hydriodic acid - nitric acid solution. Indium and titanium^{IV}, which were slightly adsorbed, yielded 3 and 2 mg, respectively. Of other elements whose behaviour had not hitherto been examined, and of which 50-mg amounts were now tested, gold, mercury^{II}, platinum^{IV} and thallium^{III} were completely held initially on the resin, but 2 mg of platinum and 50 μg of thallium as Tl^{I} were removed in the hydriodic acid - water - nitric acid treatment. Selenium^{IV} and vanadium$^{IV \text{ and } V}$ were not significantly adsorbed. The bulk of the gallium and much of the molybdenum^{VI}, tellurium^{IV} and tungsten as phosphotungstate were found in the hydrochloric acid effluent. The amounts in the hydriodic acid - nitric acid effluents were approximately 0·1, 8, 5 and 2 mg, respectively.

Results for the separation of zinc from various metals, in experiments in which the iodide treatment of the resin was used, are shown in Table I. The zinc 8-hydroxyquinolinate, from the experiments in which 5 mg of zinc were separated from 100 mg of cadmium, was tested for cadmium with 4-nitronaphthalenediazoaminoazobenzene (cadion 2B), and none (less than 5 μg) was detected.

Separation of zinc from an excess of tin—

The introduction of more tin into the zinc-containing eluate from the resin column was a disadvantage of the iodide treatment. Although some tin could be eliminated as stannic halides in preparing the solution of the sample, it was preferred to try to form a

complex that would be washed out of the resin column with the hydrochloric acid. The following simple procedure involving the use of methylarsonic acid, with which considerable complexing apparently occurs in 2 *N* hydrochloric acid solution, even though no precipitation takes place,[5] was eventually adopted. The resin was treated with 5 ml of a 5 per cent. solution of anhydrous disodium methylarsonate, 2 *N* in free hydrochloric acid, and 400 mg of the methylarsonate were also added to the solution containing tin, in which the concentration of free hydrochloric acid was similarly made 2 *N*. After transfer of the solution to the column and washing with 2 *N* hydrochloric acid as usual, only a small amount of tin remained from 50 mg initially taken. With 100 mg, the subsequent treatment with hydriodic acid caused darkening of the resin, indicative of the presence of some stannic iodide, and 3 mg of tin were eluted.

In experiments in which 50 and 5 mg of zinc were separated from 50 and 100 mg of tin, respectively, and determined in acid solution with 8-hydroxyquinoline, the errors in duplicate determinations of the larger amount of zinc were −0·27 and −0·24 mg, as compared

TABLE I

DETERMINATION OF ZINC ALONE AND IN MIXTURES AFTER ADSORPTION ON, AND ELUTION FROM, THE ANION-EXCHANGE RESIN AMBERLITE IRA-400(Cl)

Approximate weight of zinc taken, 10^{-3} g	Other metals	Approximate weight of other metals taken, 10^{-3} g	Error, 10^{-5} g	Approximate weight of zinc taken, 10^{-3} g	Other metals	Approximate weight of other metals taken, 10^{-3} g	Error, 10^{-5} g
50	—	—	−9,* −11,* 0, +2	50	Bi	50	−35†
20	—	—	−6,* −4*	5		50	+10†
5	—	—	+5,* +3*				
50	Cd	50	−27,* −13	50	Cu	50	−13†‡
20		80	−2,* +3	5		100	+14†‡
5		100	+2,* +10				
50	Sb^{III}	50	−25§	50	Fe^{III}	10	−11§
5		50	+1§	5		10	−1§
50	Sb^{V}	50	−14§				
5		50	+13§	5	Sn^{IV}	50	−24,§ −10§

* Only 40 ml of 0·25 *N* nitric acid were used.
† Corrected for contaminating iron.
‡ Corrected for contaminating copper.
§ Precipitation with 8-hydroxyquinoline in alkaline tartrate solution.

with an error of −0·54 mg in earlier experiments without methylarsonic acid, and with precipitation of zinc 8-hydroxyquinolinate in alkaline tartrate medium. The precipitates from the smaller amounts of zinc were difficult to filter off, because of contamination by hydrated stannic oxide, but virtually correct results for zinc were obtained by dissolving the precipitates and weighing the contaminant.

The presence of stannic oxide was attributed to the conversion of α-stannic compounds into β-compounds, a process induced by nitric acid, long standing and heating. When α-stannic chloride was put on a resin column and 200 mg of tartaric acid were placed in the receiver for the usual eluate, the conversion was greatly delayed. Should the eluate contain iodides, however, the oxidation to eliminate it would counteract the effect of tartrate, and if iodide were absent, the presence of tartaric acid might necessitate the application of the less desirable precipitation of zinc with 8-hydroxyquinoline in alkaline tartrate solution.

DETERMINATION OF SMALL AMOUNTS OF ZINC BY MEANS OF DISODIUM ETHYLENEDIAMINETETRA-ACETATE—

Before studying further the separation of small amounts of zinc from 100 mg of other elements, we sought a simpler means of determining quantities ranging from 5 to 0·5 mg or less, in the eluates obtained from resin columns. The versatility of disodium ethylenediaminetetra-acetate as a reagent and its value in the determination of low concentrations of metal ions[6] led to its early consideration as a substitute for 8-hydroxyquinoline. When hydriodic acid was used, the eluate (80 ml) from resin columns was 0·12, 0·12 and 0·19 *N*, respectively, in hydrochloric, hydriodic and nitric acids, and might contain in addition to zinc the maximum quantities of various elements mentioned on p. 80. The amount of tin would be reduced to 3 mg if methylarsonic acid were used. In a solution containing ammonia

and ammonium chloride, buffered to pH 10, zinc can be very satisfactorily determined directly with ethylenediaminetetra-acetate in the presence of Solochrome black as an indicator.[7] By this means, Debney[8] determined 6 μg of zinc in 2 ml of solution, and Blaedel and Knight,[9] using a high-frequency technique, determined 0·65 to 13 mg titrimetrically with a high degree of accuracy, in 80 to 100 ml of solution.

In titrations in which the colour change of Solochrome black was observed visually, it was found that, provided the rate of addition of the ethylenediaminetetra-acetate solution was adequately controlled, its zinc equivalent remained essentially constant over a range of 5 to 0·2 mg of zinc, whether the volume for titration was 100 ml or 25. End-points were more distinct in the larger volume, which was preferred. A small blank correction was made for titratable impurity in the reagents. Iodide and nitrate did not influence the results.

This simple direct titration procedure would not be applicable in the analysis of complex materials, where small amounts of other elements would accompany zinc.

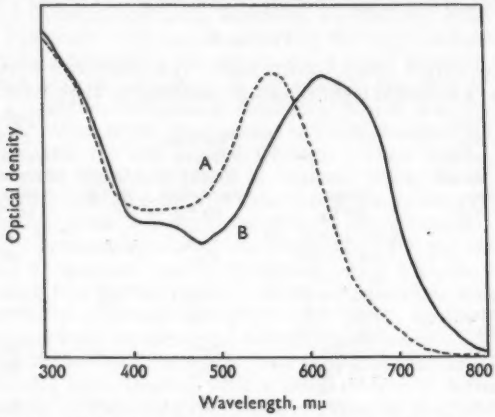

Fig. 1. Absorption spectra: curve A, zinc - Solochrome black complex; curve B, Solochrome black

An important means of improving the selectivity of ethylenediaminetetra-acetate as a reagent for zinc is to convert certain metals, *e.g.*, cobalt, copper, mercury and nickel, and zinc into complex cyanides, which do not react with ethylenediaminetetra-acetate and Solochrome black. Various uncomplexed elements, including indium, lead and manganese, may then be titrated under appropriate conditions with the reagent. If formaldehyde[10,11,12] or chloral hydrate,[13] which is easily obtained pure, be next added, the complex cyanide of zinc, but not those of the other elements cited, is broken down and excess of potassium cyanide destroyed, and the freed zinc can be titrated in the presence of Solochrome black as an indicator. Iron^{III}, which forms at pH 9 to 10 a more stable complex with Solochrome black than it does with ethylenediaminetetra-acetate, binds the indicator and must be eliminated. It may be reduced in nitrate-containing solutions by ascorbic acid[14] and effectively masked as ferrocyanide.[15]

Experiments were made to find the minimum amounts of potassium cyanide and chloral hydrate required to effect the desired reactions for 10 mg of zinc in about 100 ml of solution, such as might be obtained from resin columns. Acids were neutralised with sodium hydroxide. Various amounts of zinc were then determined by the method evolved, a correction being made for the titre found in a blank run. The ethylenediaminetetra-acetate was standardised with reference to zinc in a solution containing only ammonia and ammonium chloride.

The end-points obtained in solutions to which ascorbic acid, cyanide and chloral hydrate had been added were much less sharp than those obtained in simple buffered solutions, and, in the blank experiments, it was often difficult to decide whether or not titration was needed. Errors throughout were positive, being about 20 μg for 2 to 5 mg of zinc, but ranging from 4 to 77 μg for 0·5 mg. The results for blank runs varied erratically from 0 to 25 μg of zinc and under-estimation here probably accounted in part for positive errors elsewhere. It was decided to examine the end-point behaviour with the aid of a spectrophotometer.

Spectrophotometric evaluation of end-points—Sweetser and Bricker[16] recently determined zinc in an ammonia - ammonium chloride solution by titrating with ethylenediaminetetra-acetate and noting the change in optical density that occurred at a wavelength of 228 mμ, where the reagent absorbed strongly as compared with zinc and its complex with the titrant. In this region of the spectrum, potassium cyanide and chloral hydrate rendered the solution opaque. The absorption spectra of Solochrome black and its zinc complex were therefore next measured in ammonia - ammonium chloride solutions buffered at pH 9·5, in order to find if titration in the presence of an indicator would be possible. Recently, Karsten and

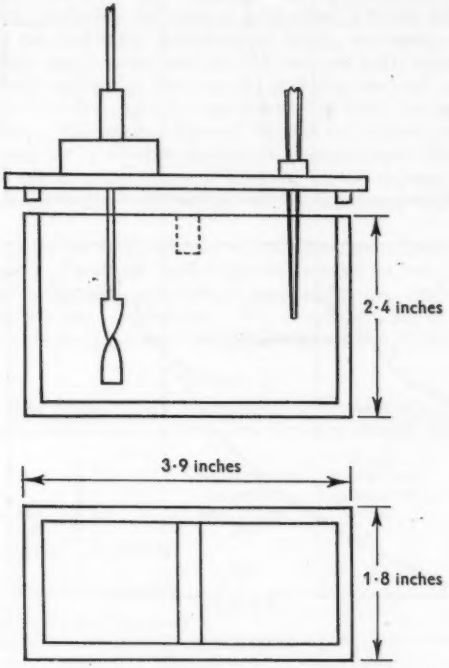

Fig. 2. Perspex titration cell and attachments

his co-workers[17] showed that magnesium could be titrated under comparable conditions at a wavelength of either 530 or 630 mμ. From the curves shown in Fig. 1 it is evident that there is sufficient difference in the optical densities at a wavelength of about 665 mμ to permit end-point determination in the titration of zinc with ethylenediaminetetra-acetate. During the titration the optical density will change only slightly until the end-point is approached, when it will increase sharply, as a result of the liberation of the indicator from its zinc complex, and reach a limiting value when liberation is complete. End-points were obtainable after the application of potassium cyanide and chloral hydrate.

As the volume for titration was expected to be about 100 ml, a Perspex cell (*cf.* Chalmers[18]), illustrated in Fig. 2, was made to fill the cell compartment of a Unicam SP500 spectrophotometer, and the compartment was fitted with a light-tight cover provided with two holes, one to accommodate a small electrically driven stirrer, the other for the insertion of the long fine tip of a microburette. Precautions were taken to exclude light at the apertures.

When various amounts of zinc, contained in ammonia - ammonium chloride solutions, were titrated with ethylenediaminetetra-acetate and correction was made for the reagent blank (3 μg of zinc), the zinc-equivalents of 1 ml of the titrant were—

Weight of zinc, μg	128	554	924	9961
Zinc-equivalent of titrant, μg per ml ..	166	167·9	168·0	168·0

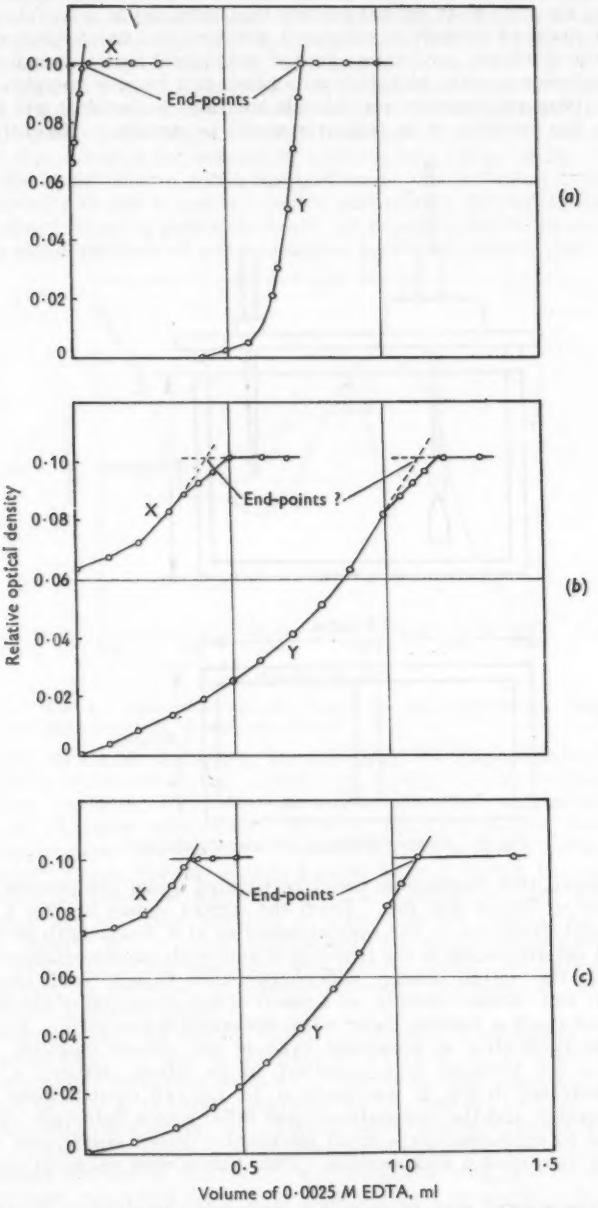

Fig. 3. Titration curves for about 100 μg of zinc (Y) and for the corresponding blanks (X)

The sharpness of the end-points in the determination of a small amount of zinc and in a blank experiment are shown in Fig. 3*a*. The above figures are to be compared with the value 167·5, averaged from four similar titrations in which 0·5-mg amounts of zinc were used and end-points were determined visually. When, however, solutions containing zinc and quantities of acids such as are obtained in eluates from resin columns were neutralised with sodium hydroxide, treated with ascorbic acid, cyanide and chloral hydrate and titrated spectrophotometrically, the errors found, after applying a blank correction of 114 μg of zinc, were −20, −62 and −58 μg on about 0·1, 1 and 10 mg of zinc, respectively. Typical end-point curves are shown in Fig. 3*b*. Further tests proved that sodium hydroxide was responsible for the errors, probably because it contained a trace of impurity that was unmasked by cyanide and reacted with Solochrome black, adversely affecting the end-point behaviour for zinc. When acids were neutralised instead with ammonia, no difficulty was experienced in interpreting end-point behaviour, and the over-all error in determining 0·1 to 10 mg of zinc in a series of six experiments where the blank value was 55 μg of zinc was $\pm$ 4 μg. In such solutions, the optical density begins to change perceptibly much farther from the end-point than in a simple ammonia - ammonium chloride solution. Nevertheless, the end-point break is quite sharp (Fig. 3*c*). In all subsequent work ammonia was used for neutralising acids before titrimetric determinations with ethylenediaminetetra-acetate.

It was desirable to find to what extent visual determinations of end-points would be improved by the omission of sodium hydroxide in neutralisations. Series of experiments were performed in which acids were neutralised with ammonia, and treatment with ascorbic acid, cyanide and chloral hydrate was given. The results shown in Table II were obtained. A set with spectrophotometric determination of end-points is included for comparison.

TABLE II

TITRATION OF ZINC WITH ETHYLENEDIAMINETETRA-ACETATE, WITH SODIUM HYDROXIDE EXCLUDED AND THE END-POINTS (EXCEPT IN (*v*)) DETERMINED VISUALLY

Remarks	Approximate weight of zinc taken, mg	Blank correction calculated as zinc, μg	Error, μg
(*i*) 5 ml of 2 *N* hydrochloric acid present initially	1·5	5	+33, +31, +27
(*ii*) Hydrochloric, nitric and hydriodic acids as from resin columns	1 to 1·3	21 $\pm$ 5	−3, −15, −1, +1, +11, −1
(*iii*) As (*ii*) but all titrations done independently of each other	0·25		−16, −6
	0·5	24	−31, +11
	2		+5
(*iv*) As (*ii*) but purer reagents	0·35	4 $\pm$ 4	+24, +38, +22, +24, +18
(*v*) As (*iv*) but spectrophotometric titration	0·35	31 $\pm$ 0	+6, +4, +7, +1

It is evident that, in visual work, errors of the order of 30 μg are possible, although precision may be good when experiments by the titration procedure are compared with one another. Spectrophotometric determination of end-points gives superior results.

INFLUENCE OF OTHER ELEMENTS ON THE DETERMINATION OF SMALL AMOUNTS OF ZINC BY MEANS OF ETHYLENEDIAMINETETRA-ACETATE—

Details concerning the amounts of various elements that could be present with zinc have been given on p. 80. In addition, 0·35-mg amounts of zinc in hydrochloric - hydriodic - nitric acid solutions were determined titrimetrically with ethylenediaminetetra-acetate by the full ascorbic acid - cyanide - chloral hydrate procedure in the presence of small amounts of some other elements whose effects were not known with certainty. Tungsten was present as phosphotungstate and tartrate was added to prevent the deposition of hydroxides of thorium and so on. The following results, corrected for the blank, are all within the limit of accuracy of the method—

Other element	Mo^{VI}	W^{VI}	Pt^{IV}	Th	Zr	Tl^{I}
Amount, mg	10	2·5	2·5	0·5	0·5	0·1
Error on zinc, μg	−5	−4	+1	+2	−2	−8

Platinum^{IV} was masked by cyanide.

Tellurium^{IV} was reduced to the element by ascorbic acid and would have to be removed. Tin, lead, manganese, gallium, indium, titanium and uranium required further consideration. None is inactivated by cyanide. Flaschka[19] showed that lead could be directly titrated with ethylenediaminetetra-acetate at a pH of 8 to 10, if tartrate were present to prevent deposition of lead hydroxide. Elements such as zinc and copper could be masked with cyanide.[5] Similar titrations of manganese[21] and indium[22] were done in hot solutions. It was essential to prevent atmospheric oxidation of manganous ions by having ascorbic acid present. Kinnunen and Merikanto[23] titrated manganese at ordinary temperature. Flaschka and Abdine[24] were unable to titrate gallium under the same conditions; its Solochrome black complex was so weak that it was necessary to add an excess of ethylenediaminetetra-acetate and titrate back with another solution. This had to be done at a pH of 8 to 9, with a lead or a manganous solution, if there was masking of zinc, etc., by cyanide. Flaschka stated that tin^{IV} did not form a complex with ethylenediaminetetra-acetate, but that it affected the indicator behaviour. Antimony caused no trouble. Under the conditions of our experiments, the titration of lead was extremely slow, and heating of the solution was impracticable in the presence of tin and with a spectrophotometric determination of the end-point. Titanium and uranium blocked the indicator. If titrations were done quickly, immediately after the addition of the indicator, 0·5 mg of titanium and the amount of uranium left after 5 mg had been submitted to the ion-exchange procedure could be tolerated, but the maximum possible amounts could not. The addition of fluoride did not prevent their interference. As tin, lead and manganese more frequently accompany zinc than do gallium, indium, titanium and uranium, an appropriate means of allowing for the first three was first sought.

DETERMINATION OF SMALL AMOUNTS OF ZINC IN ASSOCIATION WITH TIN, LEAD AND MANGANESE—

The presence of 3 mg of tin (*cf.* p. 81) considerably reduced the sharpness of the end-point in the determination of zinc, with Solochrome black as indicator. The colour-change was from lilac-grey to blue, making accurate visual detection of the end-point impossible. Spectrophotometrically, the change in optical density was one-third of the usual and spread over a larger volume of the titrant, but end-point determination was satisfactory. The presence of tartaric acid was essential to prevent deposition of stannic oxide, which caused loss of zinc. The temperature of conversion of iron into ferrocyanide had to be lowered from 70° to 40° C, and 20 minutes allowed, during which time no deposition of stannic oxide occurred. When solutions contain tin, there must be no delay in completing the experiments.

No difficulty arose in titrating manganese directly in solutions containing tartrate, cyanide and ascorbic acid. With lead, the colour change at the end-point with Solochrome black was from purple to blue. The change in optical density at the wavelength of 665 mμ was smaller than for zinc, but the end-point was sharp. Each small addition of titrant required, however, 5 minutes' waiting before a steady reading of optical density could be obtained, and a titration required 45 minutes. Titration was expedited by the addition of an excess of ethylenediaminetetra-acetate and titration of the unused reagent with a dilute standard solution of manganous chloride. Because of slight sluggishness in the latter reaction, it was desirable to have only a slight excess of ethylenediaminetetra-acetate, control of the addition of which was facilitated by adding to the solution beforehand about 100 μg of manganese^{II}, whose more distinct colour change with Solochrome black permitted the more rapid addition of ethylenediaminetetra-acetate in small excess by visual observation. Ascorbic acid had to be present in the solution to which manganous chloride was added and sufficient time allowed for reduction of any manganese^{III}, which would oxidise the indicator. In the final titration with manganous chloride, spectrophotometrically at the usual wavelength, slight blocking of the indicator occurred, without affecting end-point determination, and it could no doubt be obviated by having a little ascorbic acid in the manganous chloride solution. After the titration, the slight excess of manganous chloride necessarily added could be determined graphically. Zinc was liberated from its complex cyanide and titrated together with the excess of manganous chloride. This triple titration procedure required 30 to 40 minutes. No adverse influence of stannic oxide was noted in experiments in which a little tin was present.

The same result was obtained for 100 μg of zinc in a solution containing lead and manganese, whether a slow direct titration of these was done or the more rapid back-titration procedure was used. When the latter method was applied to the determination of zinc

accompanied by 2 mg of lead in hydrochloric - hydriodic - nitric acid solutions such as would be obtained from resin columns, and the appropriate correction was applied for the control run, the following results were obtained—

Weight of zinc taken, μg ..	464	533	5030	5144
Error, μg	+14	−7	+33	+6

If desired, the titration of manganese present in eluates from resin columns may also be done in this way. In general, when it is seen that titration is required and it is not known what element necessitates it, it is best to adopt the triple titration procedure.

Determination of small amounts of zinc in association with gallium, indium, titanium, uranium and tin—

For the determination of a small amount of zinc originally present with a large amount of gallium, indium, titanium and uranium, a simple means of separating it from the small amounts of these elements that accompany it from the resin column was sought, in order that it might be determined finally with ethylenediaminetetra-acetate. Numerous solvent extraction procedures based on the use of thiocyanate, which have been used in recent years for effecting separations of zinc from various elements, were inadequate for the present purpose. The most promising results were obtained with pyridine and thiocyanate in conjunction with chloroform as solvent.

In neutral solutions a number of bivalent metals, *e.g.*, cadmium, cobalt, copper, nickel and zinc, form complex metal pyridine thiocyanates with pyridine and ammonium or potassium thiocyanate. Zinc pyridine thiocyanate, $Zn(C_5H_5N)_2.(SCN)_2$, is soluble in chloroform. Lead may form Pb(OH)SCN, which was found to be insoluble.[25] Since the eluates from resin columns are liable to contain iodide, there is also the possibility of formation of complex pyridine iodides of, for example, zinc and antimony. Zinc pyridine iodide dissolved readily in chloroform. Antimony pyridine iodide, if extracted, would not upset the subsequent titration. The zinc from zinc pyridine thiocyanate dissolved in chloroform could be readily extracted into acid or alkaline solutions. Since acid combined with excess of pyridine dissolved in the chloroform, and pyridine salts might later influence the buffering of the solution for titration, an alkaline extractant was preferred.

In acid solutions (80 ml), neutralised to methyl red, to which ammonium thiocyanate and pyridine were added, the following results were obtained for single extractions with 10 ml of chloroform at 15° C; it was immaterial whether the original solution contained only hydrochloric acid or hydriodic and nitric acids as well—

Volume of 2 *M* ammonium thiocyanate, ml	0·25	0·25	0·25	0·1	1	2	5
Volume of pyridine, ml	0·5	1	1·5	1	1	1	1
Percentage of 2 mg of zinc extracted ..	20	45	45	55	80	95	95

In the first experiment, the pH of the residual aqueous layer was 6·7, whereas in all other experiments it was at least 7. The amount of pyridine was presumably too small.

Further experiments with 5-mg and 0·5-mg amounts of zinc, in which 2 ml of thiocyanate solution and 1 ml of pyridine were used, and two extractions with 10 ml of chloroform were made, with addition of an extra 0·5 ml of pyridine after the first extraction in order to ensure a sufficiently high pH value, gave nearly complete recovery of zinc. The efficiency of extraction was reduced by tartrate, whose adverse influence was, however, counteracted by increasing the concentration of thiocyanate and making a third extraction. An extraction apparatus (Fig. 4*a*) designed for maximum efficiency was made. The chloroform layers were run into the receiver (Fig. 4*b*), in which they were shaken up with the alkaline extractant, which was afterwards withdrawn by suction through a capillary tube into a conical flask.

Acid solutions to be submitted to solvent extraction were neutralised with ammonium hydroxide, just sufficient being added to change methyl red to yellow. An appreciable excess prevented the extraction of zinc pyridine thiocyanate.

At this time all determinations of zinc were being made with visual observation of end-points. When 0·5-mg and 5-mg amounts of zinc in 80 ml of solution were submitted to treatment with pyridine and thiocyanate and solvent extraction, and the zinc was recovered from the chloroform and determined, the errors noted, after the application of the appropriate blank corrections, averaged +20 μg and −20 μg, respectively. When similar quantities of zinc were carried through the full ion-exchange procedure with the use of hydriodic acid and methylarsonate, followed by chloroform extraction of zinc pyridine thiocyanate and so on, the corresponding errors were −13 and −60 μg. The results were as good as

could be expected with visual determination of end-points in titrations. With spectrophotometric titrations, the following results, corrected for the corresponding blank, were obtained for zinc that had been submitted to chloroform extraction of its pyridine thiocyanate complex—

Weight of zinc taken, μg ..	372	344	1329	1347	4925	4937
Blank, μg ..	25		41		35	
Error, μg ..	+6	+8	+4	+4	−17	−21

The behaviour of other metals—When put through the solvent-extraction procedure, 5 mg of gallium, 2·5 mg of indium, titanium^{IV}, bismuth or antimony, and 1 mg of uranium^{VI} imparted nothing to the chloroform. From 3 mg of tin^{IV}, the maximum amount to be expected if methylarsonic acid were used, no extraction was noted; 10 mg gave about 0·5 mg. But 2 mg of lead imparted to the chloroform a very small amount of some material that was readily titrated with ethylenediaminetetra-acetate in cyanide solution and therefore

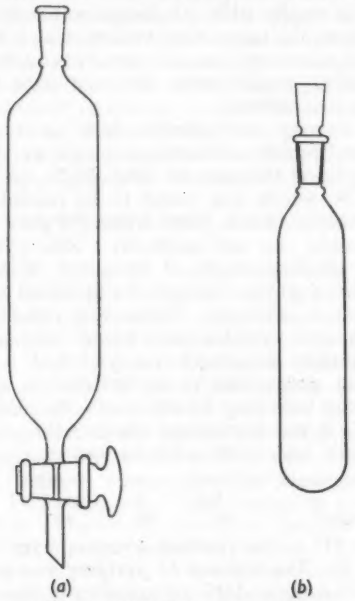

Fig. 4. Extraction apparatus

was not lead. With iron^{III}, some reduction to iron^{II} occurred, even when no ascorbic acid was used, and this, together with any manganese or copper present, would be extracted, but these elements are easily dealt with in the ensuing titration procedure.

A few mixtures containing zinc were also put through the extraction procedure and zinc was determined with the following results, control experiments being made with zinc omitted and the appropriate corrections applied—

Other metals, mg	Sn(5), Pb(2)		In(5), U(1), Ti(2·5)		Sn(5), In(5), Ti(2·5), Pb(2), U(2·5)	
Zinc taken, μg ..	432	444	342	355	4975	1930
Error, μg ..	−3	−11	−11	−11	−32	−20

Final methods for the determination of 0·5 to 5 mg of zinc in mixtures

Apparatus—

Use polythene bottles for ammonia, potassium cyanide and ethylenediaminetetra-acetate solutions and zinc-free glassware for other solutions and general operations. Erratic results may be encountered if the ethylenediaminetetra-acetate solutions are stored in glass. Calibrate volumetric ware. Columns for the resin have already been described,[1] and the solvent-extraction apparatus, which must be thoroughly cleaned before use, is shown in Fig. 4.

In this work, a Unicam SP500 spectrophotometer was used for the determination of end-points. An appropriate Perspex cell for titrations is illustrated in Fig. 2 and other necessary arrangements are described on p. 83.

REAGENTS—

Use reagents of recognised analytical grade. Distilled water should not be from a copper still. Purify and distil hydriodic acid (store under carbon dioxide) and other acids, if necessary, and prepare ammonium hydroxide solutions from cylinder ammonia. Purify and distil pyridine and chloroform. Prepare disodium methylarsonate[26] and heat it at 160° C in order to form the anhydrous salt.

Ammonium thiocyanate solution, 2 M.

Ammonium chloride solution—A 10 per cent. w/v aqueous solution.

Ascorbic acid solution—A 1 per cent. w/v aqueous solution, freshly prepared.

Potassium cyanide solution—A 5 per cent. w/v aqueous solution.

Chloral hydrate solution—A 50 per cent. w/v aqueous solution.

Disodium ethylenediaminetetra-acetate solutions, 0·008 *and* 0·0025 M.

Hydrochloric acid, 2·0 N.

Zinc chloride solution—Prepare a standard solution containing a slight excess of hydrochloric acid, either from pure metallic zinc or from freshly ignited zinc oxide. Arrange the concentration so that aliquots containing 5 or 1·5 mg of zinc can be readily taken.

Manganous chloride solution, 0·001 M.

Solochrome black indicator solution—Extract 0·2 g of Solochrome black (British Drug Houses) thoroughly with small portions of warm pyridine, filter and make up to 100 ml with pyridine. The final solution is about 0·1 per cent. and, if kept in a well stoppered glass bottle, is usable for 3 months.

PROCEDURE—

For the preparation and use of columns of resin, consult our earlier paper.[1]

The anion-exchange separation—If less than 10 mg of tin are present, apply to the resin 5 ml of a 2 *N* hydrochloric acid solution containing up to 5 mg of zinc and a maximum of 100 mg of metallic elements, and continue to add 2·0 *N* hydrochloric acid until 50 ml of solution are collected. Should the amount of tin exceed 10 mg, first treat the resin column and the sample with methylarsonate as described on p. 81, and then transfer the sample to the column and wash as usual with 2·0 *N* hydrochloric acid. In order to fix cadmium and bismuth on the resin, next apply 5 ml of 2 *N* hydriodic acid and collect the eluate in a vessel containing 200 mg of tartaric acid dissolved in a little water. Continue the elution with 15 ml of water and 60 ml of 0·25 *N* nitric acid. If the solvent-extraction procedure is required, the receiver should be the extraction funnel (Fig. 4*a*). If the solution contains tin, proceed at once with the next operation.

The formation and extraction of zinc pyridine thiocyanate, and separation from small amounts of gallium, indium, titanium, uranium, bismuth, tin and lead—If the original material contained gallium or indium, much titanium or uranium, or bismuth that was not fixed on the resin because the use of hydriodic acid was omitted, or it is desired to do a visual titration and the original material contained much tin, treat the eluate from the anion-exchange separation, which should be in the appropriate extraction funnel, as follows. If necessary, add sufficient ascorbic acid to remove free iodine, and make just alkaline to methyl red by means of ammonia. Add 5 ml of ammonium thiocyanate solution, mix and then add 1 ml of pyridine. Mix again by shaking, add 10 ml of chloroform and shake the mixture briskly for 1 minute. Allow the phases to settle and run off the chloroform layer into the back-titration tube (Fig. 4*b*) containing 2 ml of 5 *N* ammonia, 0·05 ml of ascorbic acid solution, 2 ml of potassium cyanide solution and water to make 10 ml. Repeat the extraction twice, adding to the aqueous layer in the funnel 0·5 ml of pyridine and 10 ml of solvent on each occasion. In order to recover the zinc from the organic solvent, shake the back-extraction tube briskly for 1 minute and, after the phases have separated, withdraw the aqueous layer as completely as possible, via a bent capillary with upward-pointing tip, into a conical flask containing 2 ml of ammonium chloride solution. Wash the residual chloroform thrice with 5-ml portions of water, shake, and collect the washings as before. Heat the aqueous solution to 75° C for 10 minutes in order to convert any iron^{II} that may be present into ferrocyanide, and to remove a little entrained chloroform, which would attack the Perspex cell. Proceed as described on p. 91.

Standardisation of approximately 0·0025 M *disodium ethylenediaminetetra-acetate, with spectrophotometric determination of the end-point*—If a 10-ml microburette is to be used, measure into the titration vessel an aliquot of the standard zinc chloride solution containing about 1·5 mg of zinc and raise the hydrochloric acid content to 5 ml of 2 *N*. Add ammonia to increase the pH to between 9 and 10, dilute with water to 100 ml and add 0·1 ml of indicator solution. In a rough visual experiment find the approximate position of the end-point.

Place the vessel in the cell compartment of the spectrophotometer, which should have been switched on 15 minutes earlier to warm up, and arrange the cover, stirrer and burette in position. Adjust the speed of the stirrer so that it does not affect readings. With a wavelength of 665 mμ and the switch at "check," balance the photometer bridge by adjusting the slit width, as is normally done with the solvent cell in position. Switch to "1." Add the ethylenediaminetetra-acetate solution until about 0·5 ml short of the expected end-point and, when equilibrium has been reached, measure the optical density. Continue the addition of the reagent in small measured increments, at first 0·1 ml and then, when the optical density changes rapidly, 0·05 ml. After each, record the optical density. Proceed until the latter becomes steady and finally add 0·5 ml more of the reagent, in order to check that the maximum reading has been attained. Plot optical-density measurements against burette readings and, from the edge of the plateau, determine the burette reading at the end-point. Titrate a blank containing all the reagents, but add the titrant in small increments from the start. It is not worth while to rebalance the spectrophotometer with the untitrated solution, and the titration may therefore be started at an optical density reading of, say, 0·075. During titrations, any drift in the dark current should be counterbalanced in the usual way.

For the standardisation of the 0·008 *M* ethylenediaminetetra-acetate solution, take an aliquot of standard zinc chloride solution containing about 5 mg of zinc. When the bulk of the zinc has been titrated, replace the 0·008 *M* ethylenediaminetetra-acetate solution by the standardised 0·0025 *M* solution and complete the titration as already described.

Titration of the eluates from resin columns with ethylenediaminetetra-acetate (*gallium, indium, uranium and more than* 0·5 *mg of titanium absent*)—Add to the solution 0·1 ml (0·3 ml if lead or manganese may be present) more of ascorbic acid solution than is necessary to decolorise any iodine present. If lead may be present, add about 2 ml of manganous chloride solution. Next add sufficient 5 *N* ammonia to raise the pH to between 9 and 10, followed by 1 ml of potassium cyanide solution. Heat at 40° C for 20 minutes (or at 70° C for 5 minutes if tin is absent) in order to form ferrocyanide, cool quickly and add 0·1 ml of indicator solution. Proceed as described under (*a*) or (*b*).

(*a*) *The colour of the solution is blue* (*lead and manganese absent*)—Add 1 ml of chloral hydrate solution, transfer the solution to the titration cell and arrange for titration spectrophotometrically. Balance the instrument with the switch in the "check" position, then switch to "1," set the optical-density scale at 0·005 or 0·01 and, with the dark-current shutter open, add the ethylenediaminetetra-acetate solution slowly, with stirring, until the galvanometer balances. Close the dark-current shutter, re-adjust the dark current if necessary and, after re-opening the dark-current shutter, continue the addition of the titrant in small increments, as already described, until the end-point is reached and then exceeded. Carry out a similar titration on a blank which has been subjected to the full procedure. Determine the end-points in the usual way.

If the amount of zinc is known to be large, use the more concentrated solution of ethylenediaminetetra-acetate for much of the titration, but obtain the end-point by titration with the less concentrated solution.

If tin is present, titrate very cautiously, as the end-point reaction is slow and the change in optical density is only one-third of the normal. Up to 0·5 mg of titanium may be tolerated only if titration is carried out rapidly after the addition of the indicator.

(*b*) *The colour of the solution is pink* (*lead or manganese or other titratable element present*)—Check that sufficient potassium cyanide has been added to mask zinc. If lead is known to be present and manganous chloride has been added, titrate very slowly with 0·0025 *M* ethylenediaminetetra-acetate until the colour is seen to be permanently blue and an excess of about 0·2 ml has been added. The excess need not be accurately known. Transfer the solution to the titration cell, assemble in the spectrophotometer as before and arrange for back-titration, at the usual wavelength, with the standardised manganous chloride solution. Switch to "1," set to an optical density of 0·1 and balance

the instrument by means of the slit and sensitivity controls. Add the titrant slowly in small increments until the optical density starts to decrease, after which reduce the size of the increments and wait longer before measuring optical density. Do not go further than necessary to locate the end-point. From the graph, determine the end-point and the excess of manganous chloride added.

For the determination of zinc add 1 ml of chloral hydrate solution and proceed as already prescribed. Correct for the excess of manganous chloride and for the relevant blank experiment. Standardise the manganese chloride solution by adding a known excess of ethylenediaminetetra-acetate solution to a titrated blank solution and titrating with the manganese solution.

In the absence of lead, a pink solution at the cyanide stage may, alternatively, be titrated directly with ethylenediaminetetra-acetate by the spectrophotometric technique.

Titration of zinc with ethylenediaminetetra-acetate after the solvent-extraction procedure—Transfer the cooled, chloroform-free solution (p. 89) to the titration cell and add 0·1 ml of Solochrome black solution. If the colour is not blue, titrate directly with the reagent by the spectrophotometric method until it is. Since lead has been removed, slowness in titration is not expected. If the colour is blue or has been brought to blue by titration, add 2 ml of chloral hydrate solution and titrate the liberated zinc in the usual manner. Approach the end-point cautiously, as it is sharper than for a normal titration where cyanide and chloral hydrate have been used. Correct for the full extraction blank. The time required for solvent extraction, back-extraction and titration is 30 to 35 minutes.

Preparation of alloy solutions—Treat 100 mg of alloy with hydrochloric acid and the minimum of 100-volume hydrogen peroxide or of nitric acid, excess of which should be removed by evaporation to small volume in the presence of an excess of hydrochloric acid. Dissolve the residue in a minimum of hot 2 *N* hydrochloric acid; then, if lead chloride is present, cool to 0° C and separate the latter, together with any insoluble matter, by centrifuging. If more than 10 per cent. of tin may be present, add 400 mg of disodium methylarsonate and make the solution 2 *N* in free hydrochloric acid. Transfer the solution and the appropriate washings with 2 *N* hydrochloric acid to a suitably prepared resin column. Proceed as required.

Preparation of a solution from borosilicate glass—To a 100-mg sample add a few drops of water and 0·5 ml of 40 per cent. w/v hydrofluoric acid and evaporate as far as possible to dryness on a hot-plate with a steady surface temperature of 100° C, stirring occasionally with a platinum wire. Add to the residue 0·1 ml of water, 0·25 ml of hydrofluoric acid and 0·1 ml of 60 per cent. w/w perchloric acid, and re-evaporate, with final gradual increase in the temperature, to expel perchloric acid. Reheat with 0·2 ml of perchloric acid until this acid is removed. Dissolve the residue in 5 ml of 2 *N* hydrochloric acid and proceed to the anion-exchange separation, using only hydrochloric and nitric acids, and the titration of zinc (spectrophotometric determination of end-point) via the ascorbic acid - potassium cyanide - chloral hydrate method.

Results

The methods prescribed were applied to the determination of zinc in synthetic mixtures and in various materials, with the results shown in Tables III and IV, respectively. All the results for zinc in mixtures refer to weight aliquots of standard solutions of zinc chloride prepared from Hilger's "H.H.P." zinc (99·99 per cent.). Many of the additional elements were derived from Specpure solutions or from similar high-quality solids. Control experiments with the other elements were made without addition of zinc chloride in order to allow for traces of titratable impurity. The results for these seldom differed significantly from those for the associated blank experiments. The blanks were commonly of the order of 50 μg of zinc.

The effects of various acid radicals were examined, as the corresponding acids might be required or encountered in preparing solutions of materials, and it would be an advantage if small amounts could be tolerated in the anion-exchange process.

In the experiment with 5 mg of uranium an end-point was obtained on titrating rapidly but the titrated solution soon became pink again as a result of blocking of the indicator. A duplicate of the experiment with cobalt, nickel and manganese gave no end-point, supposedly because of accidental oxidation of the indicator by manganese[III].

The mean error for the 48 experiments with about 0·5 mg of zinc is -1 μg and for the 21 with 5 mg of zinc $+4$ μg. The corresponding standard deviations are 8 and 15 μg.

TABLE III

TITRIMETRIC DETERMINATION OF ZINC IN SOLUTIONS AFTER SEPARATION BY ANION EXCHANGE

Approximate weight of zinc taken, 10^{-3} g	Approximate weight of other elements or radicals, 10^{-3} g	Error, 10^{-6} g	Approximate weight of zinc taken, 10^{-3} g	Approximate weight of other elements, 10^{-3} g	Error, 10^{-6} g
(a) *Elution with water and nitric acid only*—					
5	—	+17, −24	0·5	Cu, 100	+1, +5
0·5	—	0, −5	0·5	Be, 5	+6
5	SO_4'', 250	+20	0·55	Be, 100	+3
0·4	SO_4'', 250	+21	0·5	Cr, 5	+3
5	PO_4''', 50	+8	0·6	Cr, 100	+7
0·4	PO_4''', 50	−6, 0	0·5	Th, 5	+3
5	AsO_4''', 50	+3	0·55	Th, 100	+2
0·5	AsO_4''', 50	−6	0·5	Zr, 5	−10
5	AsO_3''', 50	+1	0·5	Ti, 5	−9
0·55	AsO_3''', 50	−13	0·45	Zr, 50; Ti, 50	+7, +5
5	NO_3', 50; ClO_4', 50	−3	0·5	U, 5	+7
0·5	NO_3', 50; ClO_4', 50	+6	0·5	Co, 20; Ni, 40; Mn, 40	+1
(b) *Elution with hydriodic acid, water and nitric acid*—					
5	Cd, 100	+5, +10	5	Bi, 50	−2, +4
0·5	Cd, 100	−5, +7	0·4	Bi, 50	−7, −8
5	Pb, 100	+26, +36	0·4	Al, 100	−8, +3
0·4	Pb, 100	−3, 0			
5	Sb^{III}, 50	+13, +8	0·4	Fe^{III}, 100	+13, +8
0·4	Sb^{III}, 50	−7, −9			
5	Sb^{V}, 50	+11, +6	0·5	Au, 25, Pt, 25,	−24, −9
0·4	Sb^{V}, 50	−9, +5		Hg^{II}, 25, Tl^{III}, 25	
(c) *Methylarsonate treatment and elution as in* (b)—					
5	—	−12, +2	5	Sn, 100	−31, −7
0·5	—	+1, +10	0·4	Sn, 100	−11, −3
(d) *Elution as in* (a), *followed by the solvent-extraction procedure*—					
0·5	Ga, 100	+1, +3	0·5	U, 100	−7, −5
0·5	In, 100	−4, −8			

TABLE IV

DETERMINATION OF ZINC IN ALLOYS AND IN A GLASS

Alloy	Approximate composition of material: Al, %	Cu, %	Sn, %	Pb, %	Sb, %	Fe, %	Ni, %	Zn, %	Zinc found, %
Aluminium alloy No. 181 ..	87	5	⩽1	2	—	<1	2	2·16 to 2·54*	2·41, 2·42
Aluminium alloy	89	2	6	—	—	<1	—	0·80†	0·94, 0·94
Bronze No. 183	—	83	10	2	⩽1	⩽1	—	1·72 to 2·00*‡	1·88, 1·89
White metal No. 177 ..	—	<1	5	83	12	⩽1	—	0·02 to 0·16*	0·03, 0·03
White metal No. 178 ..	—	4	84	4	8	⩽1	—	0·31 to 0·47*‡	0·44, 0·44

	SiO_2, %	Al_2O_3 + Fe_2O_3, %	BaO + CaO, %	Na_2O + K_2O, %	B_2O_3, %	ZnO, %	ZnO, %
Borosilicate glass	65	5	4	9	15	1·86§	1·89, 1·87

* British Chemical Standard, range in certificate values.
† Spectrographic determination by the North British Rubber Company.
‡ The methylarsonate treatment was used.
§ Value supplied by Research Laboratories of The General Electric Company.

respectively. It is evident that 50-mg amounts of zinc would be determined with adequate accuracy and precision if, after ion exchange, an aliquot of the eluate from the resin, containing 5 mg of zinc, were titrated spectrophotometrically. It is highly probable, however, that visual titration of larger amounts of zinc with a more concentrated solution of ethylenediaminetetra-acetate will suffice.

In the analyses of the British Chemical Standard samples, all the results fall within the range of the certificate values and precision is excellent. It is obvious that the prescribed methods have a wide range of applicability.

One of us (J. A. H.) is grateful for the award of an Edinburgh University Studentship. We are indebted to Mr. R. C. Chirnside, of the Research Laboratories of The General Electric Company, Wembley, and to the North British Rubber Company, Edinburgh, for analysed samples, and also to the Geigy Company, Manchester, and Versenes Incorporated, Framingham, Mass., U.S.A., for samples of chelating reagents. We thank Dr. H. Flaschka of Graz, Austria, who gave us valuable pre-publication information about the use of ascorbic acid for reducing iron^{III}.

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Discussion

Dr. R. J. Magee asked how germanium behaved in the anion-exchange procedures and whether it interfered in the titrations.

Mr. Hunter replied that some germanium was adsorbed and eluted along with the zinc, but since it could be removed by volatilisation as germanium tetrachloride, it was not examined in titration.

Dr. F. J. Elliott asked whether the method was likely to be applicable to the determination of zinc in biological materials.

Mr. Hunter said that, after wet-ashing with, say, perchloric acid, the method could be directly applicable. Perchlorate, sulphate, nitrate and phosphate did not interfere with the adsorption of zinc.

Dr. Magee asked how arsenic, antimony and tin behaved during anion exchange and whether they had been eluted and separated.

Mr. Hunter replied that arsenic was not held at all, all the antimony^{III} was held, 75 per cent. of the antimony^{V} and all the tin^{IV}, from 2 *N* hydrochloric acid solutions. Methylarsonic acid prevented adsorption of much tin^{IV}. No method for complete elution of antimony or of tin had been found, but change in the concentration of hydrochloric acid might help.

Dr. A. Magnus Pyke asked if polarography had been considered for determining the small amounts of zinc.

Mr. Hunter said it had, but for about 5 mg, an accuracy of $\pm$ 10 μg, being 1 in 500, could hardly be expected from polarography. Further, the small amounts of various other elements liable to accompany zinc would be difficult to eliminate in a polarographic procedure.

Mr. J. A. Eggleston asked if the method was likely to be usable for amounts of zinc less than 50 μg, and whether, with smaller amounts of zinc, the blank on the reagents would not be appreciable.

Mr. Hunter replied that if the blank could be lowered, the limit of determination of zinc could be taken somewhat below 50 μg, and the lower limit would then be governed by the over-all accuracy of $\pm$ 10 μg.

The Estimation of Vitamin-B_{12} Activity in Feeding Stuffs with *Lactobacillus leichmannii* and *Ochromonas malhamensis*

By D. H. SHRIMPTON*

(*Presented at the meeting of the Biological Methods Group on Friday, May 13th*, 1955)

The vitamin-B_{12} activity of some animal by-products used as feeding stuffs has been determined with *Lactobacillus leichmannii* and *Ochromonas malhamensis*. A description is given of the microbiological methods; these have been adapted to determine the vitamin-B_{12} activity of feeding stuffs with as little manipulation as possible. A semi-automatic syringe used for dispensing solutions is described, and also a shaker of simple design, in which cultures of *O. malhamensis* were grown.

All but one of the feeding stuffs had different vitamin-B_{12} activities for each of the test micro-organisms. The activities for *O. malhamensis* were less than or similar to the activities for *L. leichmannii*, and it was concluded that at best *O. malhamensis* was no better than *L. leichmannii* in predicting the vitamin-B_{12} activity of feeding stuffs for chicks.

It is known that many biological materials contain substances related to vitamin B_{12}, which, although they cannot be converted to cyanocobalamin by treatment with cyanide, show a vitamin-B_{12}-like activity for many micro-organisms and animals. In many cases, however, the relative activity of these substances differs according to the micro-organism or animal used for the assay of vitamin B_{12}.[1,2] In mixtures of these many growth factors only cyanocobalamin, at present, may possibly be determined specifically.[1] In the absence of further evidence it is convenient to attribute vitamin-B_{12}-like activity for *Ochromonas malhamensis* to cyanocobalamin and activities for other micro-organisms and test animals to vitamin-B_{12}-like substances, which may or may not be cyanocobalamin. A question that must therefore be posed is: can the vitamin-B_{12} activity of a feeding stuff for animals be predicted from its content of cyanocobalamin? As a contribution to the solution of this problem, the cyanocobalamin content of several feeding stuffs has been determined and also the vitamin-B_{12} activity for *Lactobacillus leichmannii* of the same samples. A detailed description of the methods used to obtain these values is reported in this paper and the results are discussed with particular reference to the vitamin-B_{12} activity of feeding stuffs for chicks.

Methods

Vitamin-B_{12} activity has been determined microbiologically. Although many workers have developed techniques of assay that are suited to their particular problems, there is no published account of the techniques used in assaying feeding stuffs. A description of the methods used in this work therefore follows.

Culture of the test micro-organisms—

L. leichmannii ATCC4797 was cultured in a single-strength basal medium,[3] modified by the addition of 40 mg of asparagine[4] to every 100 ml and by replacing L-cystine with L-cysteine.[4] A commercial casein hydrolysate (Allen & Hanbury Ltd.) was used. The organism was maintained in stab cultures of this medium containing 3 per cent. w/v of agar and supplemented with 0·5 μg of standard vitamin B_{12} (Cytamen, Glaxo Laboratories Ltd.) to every 100 ml. The organism was subcultured once a fortnight, first into a liquid culture of similar composition but containing no agar, and then into a fresh stab culture, which was incubated at 37° C for 16 hours and then stored at 1° C. An inoculum for use in an assay was prepared in two stages. Ten millilitres of the modified single-strength basal medium were supplemented with 50 mμg of standard vitamin B_{12}, inoculated from the stock stab culture and incubated in a 50-ml centrifuge tube at 37° C for 48 hours. The culture was centrifuged and resuspended in 10 ml of physiological saline. A second similar tube of

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medium, but supplemented with only 0·5 mμg of standard vitamin B_{12}, was then inoculated with one drop of the suspension and incubated at 37° C for 16 hours. The culture was centrifuged and resuspended in physiological saline, one drop being used to inoculate each tube prepared for assay. An inoculum of a "depleted" culture of this type gave a low blank, but nevertheless grew vigorously in the presence of vitamin-B_{12}-like growth factors.

O. malhamensis was grown in the medium described by Ford.[1] One drop of this dark green-brown culture was used to inoculate each tube prepared for assay.

STANDARD VITAMIN B_{12}—

A 1-ml ampoule of Cytamen 50 (Glaxo Laboratories Ltd.) containing approximately 50 μg of vitamin B_{12} was diluted to 10 ml and stored at +1° C for up to 1 month. The concentration of vitamin B_{12} in this dilute solution was determined by measuring $E^{\text{g per litre}}_{1\text{ cm}}$ at 361 mμ and comparing this with a reference value[5] for $E^{\text{g per litre}}_{1\text{ cm}}$ of 20·4. The concentration of vitamin B_{12} was found to be 5·30 μg of the vitamin per ml of solution.

PREPARATION OF SAMPLES FOR ASSAY—

The procedure of Scheid and Schweigert,[6] in which 1 g of the finely ground sample was heated in an autoclave at 15 lb per sq. in. for 30 minutes with 20 ml of a 1 per cent. w/v solution of sodium acetate at pH 4·5, was modified by the inclusion of potassium cyanide in the acetate solution at 0·01 per cent. w/v. Two extracts were prepared for each sample.

The extracts were centrifuged and the supernatant liquid was diluted so that it contained a vitamin-B_{12} activity approximately equivalent to 0·1 mμg of the vitamin per ml of extract.

When the vitamin-B_{12} activity was determined with *O. malhamensis*, a similar extraction procedure was followed, modified only by the exclusion of sodium acetate. Ford[1] reported that the presence of more than 0·1 per cent. of sodium acetate in the growth medium markedly inhibited the growth of this organism.

With some materials, but not with those described below, it may be necessary to modify this extraction procedure. Thus, for example, when sows' milk is assayed, digestion with papain is necessary,[7] and with some balanced rations the temperature of extraction must be kept below 100° C to prevent turbidity in the extract.

PREPARATION OF ASSAY TUBES—

Wire baskets, each holding 70 rimless tubes (19 mm × 120 mm), were boiled for 10 minutes in a 2 per cent. w/v solution of a proprietary glass cleanser (Brylyanz, obtainable from G. T. Gurr Ltd., London, S.W.6). Each tube, while remaining in the basket, was rinsed three times each with tap water and distilled water and then placed in a drying oven. This method was adopted in place of two other methods that had previously been used. When the tubes were washed with chromic acid, occasional erratic results were obtained. The impression given was that of a cumulative effect, and it is possible that in older scratched tubes the chromic acid was not always removed by subsequent rinsing. Soda washing followed by an acid rinse was satisfactory but laborious.

ASSAY PROCEDURE—

It has frequently been observed in this laboratory that results calculated from replicate sets of assay tubes of a single extract were in better agreement than those calculated from replicate sets of assay tubes from two similar extracts of the same material. Also, results calculated from replicates assayed at the same time were in better agreement than when they were calculated from replicates assayed on different occasions. The standard procedure adopted has therefore been to prepare two extracts of each material to be assayed and to set up duplicate sets of assay tubes for each extract. The entire procedure was then repeated with fresh extracts. If the four results obtained differed from their own mean by more than 10 per cent., the determination was repeated.

The vitamin-B_{12} activities of feeding stuffs reported below are each the mean of four results that differ from their respective means by less than 10 per cent.

Duplicate sets of assay tubes were filled, as shown in Table I, for each of the two extracts prepared from each sample. A triplicate set of assay tubes containing graded levels, up to 0·5 mμg per tube, of standard vitamin B_{12} was also prepared. The total volume contained in each tube was always 10 ml when *L. leichmannii* was the test organism and 5 ml when

O. malhamensis was the test organism. The extract and water and, when *O. malhamensis* was the test organism, the basal medium were dispensed with a Hauptner 30-ml capacity syringe, illustrated in Fig. 1. This instrument delivered 1, 2, 3, 4 or 5 ml at a time with an error of less than 1 per cent. and was supplied by Messrs. Willows Francis Ltd. (73, 75 and 89*a*, Shacklewell Lane, London, E.8). When *L. leichmannii* was the test organism, the basal medium was dispensed with an automatic syringe of the type used for preparing bacterial plates. This instrument delivered 5 ml at a time with an error of less than 2 per cent.

The 20 tubes necessary for the assay of two extracts of one sample were arranged in one rack numbered in one corner so that marking of individual tubes was unnecessary.

TABLE I

QUANTITIES OF HYDROLYSATE, STANDARD, WATER AND BASAL MEDIUM DISPENSED INTO ASSAY TUBES

	With *L. leichmannii* as test organism					With *O. malhamensis* as test organism				
Tube	A	B	C	D	E	A	B	C	D	E
Hydrolysate, ml ..	1	2	3	1	1	1	2	3	1	1
Standard, ml ..	–	–	–	1	2	–	–	–	1	2
Water, ml	4	3	2	3	2	3	2	1	2	1
Basal medium, ml ..	5	5	5	5	5	1	1	1	1	1
Total volume, ml ..	10	10	10	10	10	5	5	5	5	5

When *L. leichmannii* was the test organism, the tubes were covered with metal caps (Oxo Ltd.) and steamed for 30 minutes. After cooling and inoculation the tubes were incubated in a constant-temperature room at 37° C for 48 hours.

When *O. malhamensis* was the test organism, the tubes were covered with glass specimen tubes and heated in an autoclave at 10 lb per sq. in. for 10 minutes. After cooling and inoculation the tubes were placed in a specially constructed shaker of simple design, shown in Fig. 2, and incubated in a constant-temperature room at 30° C for 96 hours. The shaker consisted of a box, constructed of $\frac{3}{4}$-inch thick wood, which was suspended on four spring-steel strips from a steel gantry. A steel roller on one side of the box was struck by a steel striker approximately once every two seconds. The striker was turned by a NECO single-phase $\frac{1}{3}$-h.p. induction motor, type R.I. (Normand Electrical Co. Ltd., Neco Works, London, S.W.4), which was cooled by a fan and had a built-in gear box to give a drive of 35 r.p.m.

At the end of incubation the tubes were steamed for 30 minutes, cooled and shaken. The suspensions were transferred to special tubes before their turbidities were determined in an EEL nephelometer (Evans Electroselenium Ltd.).

COMPUTATION OF RESULTS—

The turbidities were plotted against the corresponding volumes of standard vitamin B_{12} or of extract, and the mean slope ratios were calculated according to the principle of Kodicek and Pepper,[8] by using their internal-standard method. The results were always plotted and the curves inspected. If the response curves were linear, their mean gradients were calculated as follows. Where A, B, C and D were the mean turbidities of solutions containing 1, 2, 3 and 4 ml of standard vitamin B_{12}, respectively, the mean slope of the standard response curve was—

$$\frac{6B + 3C + 2D - 11A}{18}; \text{ let this be equal to } z.$$

Also, where a, b and c were the mean turbidities corresponding to solutions containing 1, 2 and 3 ml of extract, respectively, and where d and e were the mean turbidities corresponding to 1 ml of extract with the addition of 1 or 2 ml of standard vitamin B_{12}, respectively, the slopes of the extract curve and of the internal-standard curve were—

$$\frac{2b + c - 3a}{4}; \text{ let this be equal to } x, \text{ and}$$

$$\frac{2d + e - 3a}{4}; \text{ let this be equal to } y.$$

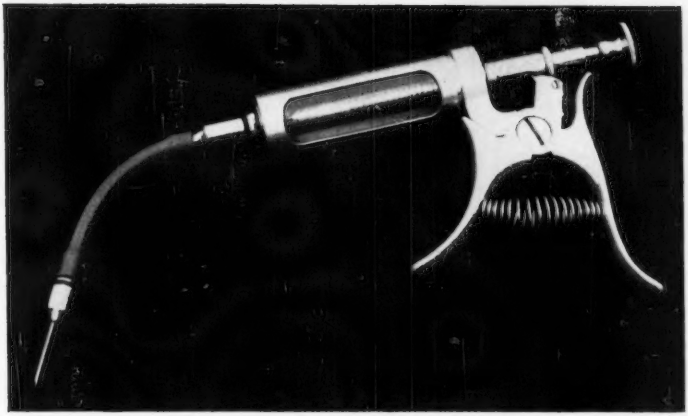

Fig. 1. Hauptner 30-ml capacity syringe

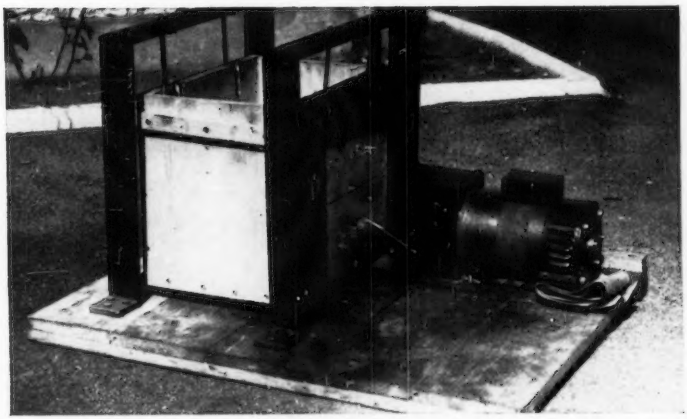

Fig. 2. Specially constructed shaker used for determining vitamin-B_{12} activity with *Ochromonas malhamensis*

[To face p. 96

The vitamin-B_{12} activity of the extract in μg per g was therefore given by—

$$\frac{x}{y} \times \frac{[\text{standard vitamin } B_{12}]}{[\text{extract}]}.$$

The ratio of the slopes of the internal standard and standard curves was given by y/z, and when this was between 0·9 and 1·1 the assay was considered valid. When the response curves were not linear over the whole of the range, the calculation was modified so that only these points within the linear portion of the response were used. A value so obtained was checked with a further assay whenever possible.

Since the calculation of potency was made only from the results of the internal standard and the extract, the assay was basically of Wood's "common-zero five-point" design,[9] the zero in this case being the growth response resulting from 1 ml of extract.

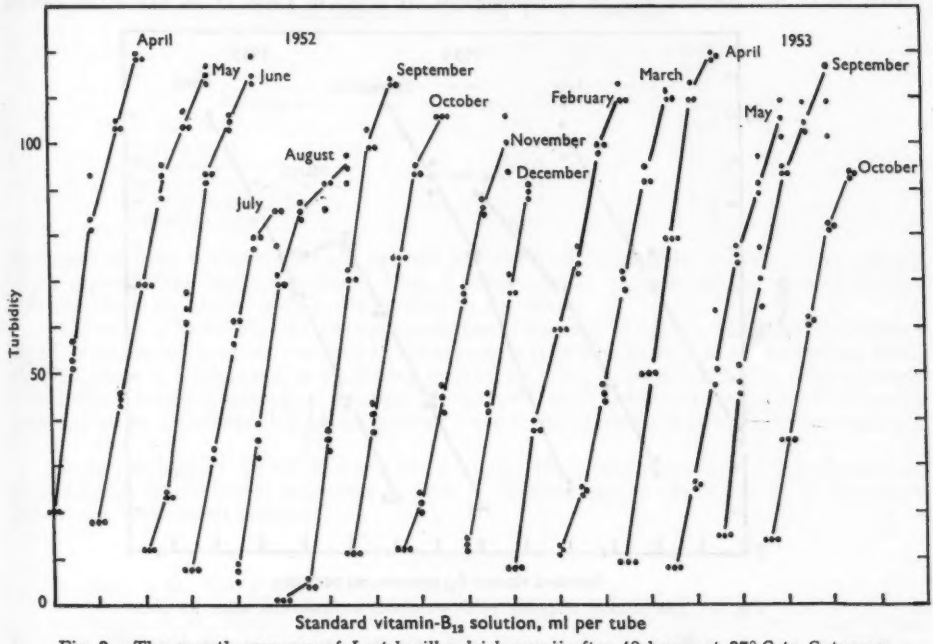

Fig. 3. The growth response of *Lactobacillus leichmannii* after 48 hours at 37° C to Cytamen vitamin B_{12}

FEEDING STUFFS ASSAYED—

The vitamin-B_{12} activities have been determined of the following feeding stuffs: herring meal, white-fish meal, whale-meat meal, meat and bone meal and dried skim milk. They are used in Great Britain as protein supplements, and whenever possible the sample assayed was from a batch for which the processing history was known.

RESULTS AND DISCUSSION

GROWTH OF MICRO-ORGANISMS—

The standard curves of both *L. leichmannii* and *O. malhamensis* were linear over part of their ranges. A series of such standard curves obtained over a period of several months is shown for *L. leichmannii* in Fig. 3 and for *O. malhamensis* in Fig. 4. Three tubes were used at each level, and when two or more points are coincident they are placed side by side. Since a straight-line response was obtained consistently, no transformations of the type recommended by Clarke,[10] log of dose against response, or Wood,[11] log of dose against log of response, were made.

When *L. leichmannii* has been the test organism, a series of successful assays has sometimes been followed by a period in which growth was either slight or excessive at all levels

of standard. It has always been possible to correct failures of this type by growing a depleted inoculum from a freeze-dried culture obtained from the National Collection of Industrial Bacteria and repeating the assays. When the growth has been erratic, several steps in the assay procedure have, at different times, been suspect, but it has not been proved that any one of these has been responsible.

With *O. malhamensis* as the test organism, it has been found that growth was sometimes unsatisfactory when the assay tubes were covered with metal caps while they were shaken. It seemed probable that metal filings were falling into some of the tubes, resulting in a blackening of the cultures. There has been no difficulty with glass covers.

VITAMIN-B_{12} ACTIVITY OF FEEDING STUFFS—

The vitamin B_{12} activities, expressed as μg of standard vitamin B_{12} per g of dry feeding stuff, for *O. malhamensis* and for *L. leichmannii* are given in Table II, in which the feeding

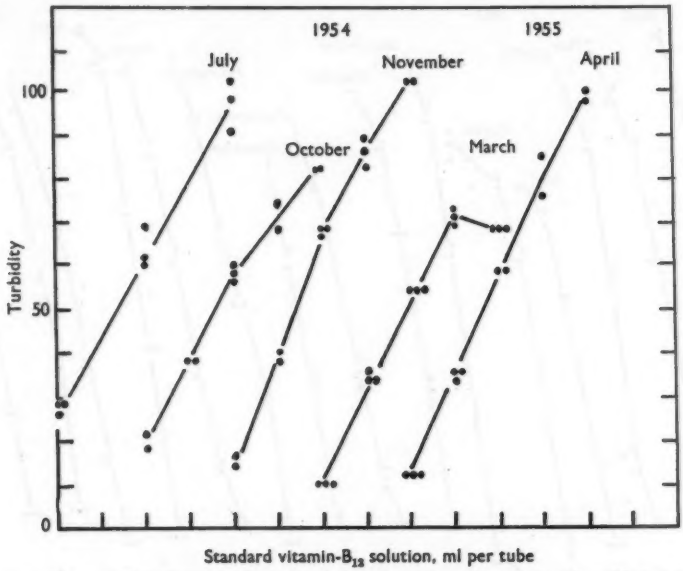

Fig. 4. The growth response of *Ochromonas malhamensis* after 96 hours

stuffs are arranged in descending order of activity for *O. malhamensis*. In only herring meal was the activity similar for both micro-organisms, when the activities differed from their mean by less than 10 per cent. Dried skim milk had a greater activity for *O. malhamensis*, which was an unexpected result in view of the believed specificity of this organism for cyanocobalamin. However, the difference is not large and it is possible that the activity for *L. leichmannii* might have been suppressed. The remaining three meals each had an appreciably greater activity for *L. leichmannii*, white-fish meal having more than twice the activity for *L. leichmannii* that it had for *O. malhamensis*.

The similarity of the vitamin-B_{12} activities of herring meal for the two micro-organisms is surprising in view of the differences found in the other feeding stuffs and most particularly in white-fish meal. Ford[1] also has reported that the vitamin-B_{12} activities of fish solubles, presumably prepared from herring, for *O. malhamensis* and for *Escherichia coli* were identical.

It has been frequently stated that animal by-products, such as those evaluated in this work, contain unidentified growth factors. Possibly owing to the absence of any method of assaying such factors, it has been customary to refer to growth effects of a vitamin-like nature for chicks as those due to the "vitamin-B_{12}" content of the feeding stuffs. Unfortunately, there are many difficulties in biological assays of the vitamin-B_{12} activities of feeding stuffs for chicks,[2] and they are not suitable for routine work. At present, therefore, the analyst of feeding stuffs designed for animals must make some prediction of the vitamin-B_{12}

activity of that feeding stuff for an animal, even though it is possible that only cyanocobalamin can be determined specifically.

Denton, Lillie and Sizemore[12] have studied the correlation between vitamin-B_{12} activity of animal by-products for *L. leichmannii* and for the chick. They reported agreement between the methods for a number of feeding stuffs including herring meal, steam fish meal, whale solubles and dried skim milk. When the methods gave results that were not in agreement, the activity for the chick was greater than for *L. leichmannii* (excepting those from sewage sludge preparations, for which the activity for *L. leichmannii* was the higher). Thus, a closer determination of the vitamin-B_{12} activity of a feeding stuff for chicks would only

TABLE II

THE VITAMIN-B_{12} ACTIVITIES OF FEEDING STUFFS FOR *O. malhamensis* AND FOR *L. leichmannii*

Feeding stuff	Standard vitamin-B_{12} activity per g of dry feeding stuff for	
	O. malhamensis, μg	*L. leichmannii*, μg
Herring meal	0·16	0·18
Whale-meat meal	0·053	0·092
Meat and bone meal	0·048	0·061
Dried skim milk	0·036	0·028
White-fish meal	0·028	0·061

be expected from a microbiological method for which the calculated activities were similar to or greater than those calculated from *L. leichmannii*. *O. malhamensis* has not fulfilled this condition for the feeding stuffs studied in this work.

Although a knowledge of the cyanocobalamin content of feeding stuffs as determined by *O. malhamensis* is of interest, the micro-organism is at best no better, in the feeding stuffs studied, than *L. leichmannii* in predicting their vitamin-B_{12} activity for chicks. The reported correlations between assays of vitamin-B_{12} activity with chicks and with *L. leichmannii* may indeed be fortuitous, but at the present state of development it is nevertheless valuable.

I am indebted to Dr. J. E. Ford for a culture of *O. malhamensis*. It is a pleasure to acknowledge the technical assistance of Miss K. Ramsay and to thank Dr. K. J. Carpenter for advice and useful criticism.

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Determination of Quaternary Ammonium Compounds as Phosphotungstates

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Surface-active quaternary ammonium compounds are shown to be quantitatively precipitated by phosphotungstic acid, whereas alternative gravimetric methods give inaccurate results with a number of such compounds. By weighing the quaternary phosphotungstate after drying and again after ignition, it is possible to calculate the amount of quaternary cation in an unknown sample and also the ionic weight of the quaternary salt. All alternative methods of analysis hitherto proposed require the molecular weight of the quaternary compound to be known before the amount present in a sample can be determined, leading to a vicious circle of approximations, particularly with unknown materials.

Interferences are discussed and general techniques by which these may be overcome are suggested.

For the determination of quaternary ammonium compounds several methods that make use of precipitation reactions with complex anions have been proposed. Among the anions that have been used may be mentioned ferrocyanide,[1] ferricyanide,[2] dichromate[3] and reineckate.[4,5] These methods are limited in application, since the reactions are not always stoicheiometric, particularly with cations of low equivalent weight.[6] Further, when calculating the results of such determinations the molecular weight of the quaternary must be known, whereas in general the molecular weight of the compound is unknown or at best assumed.

Lematte, Boinot, Kahane and Kahane[7] used phosphotungstic and silicotungstic acids to assay low molecular weight quaternary ammonium compounds and, although it has been shown that surface-active quaternary ammonium compounds are precipitated by these reagents,[8,9] no use has been made of the reaction for their quantitative determination. The present investigation shows that they are quantitatively precipitated as phosphotungstates. (A summary of some of the results has been reported previously.[6])

Ignition of the quaternary ammonium phosphotungstate at dull-red heat results in an inorganic residue free of organic matter[7]—

$$Q_3PO_4.12WO_3 \rightarrow HPO_3.12WO_3 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (A)$$

This residue may be dehydrated at higher temperatures as follows—

$$2HPO_3.12WO_3 \rightarrow P_2O_5.24WO_3 + H_2O$$

Such dehydration corresponds to a loss in weight of only three parts per thousand and is therefore unlikely to introduce a serious error.

Experimental

Standard quaternary ammonium compound solutions, 0·01 *N*, were prepared by dissolving the required amounts of chemically pure materials (which are available, some in limited amounts, from Milton Industrial Chemicals (London) Ltd. and by arrangement through most chemical supply houses) in water and standardising with 0·01 *N* silver nitrate by the method of Dubois.[10]

A 20-ml sample of the 0·01 *N* quaternary ammonium compound solution was diluted to approximately 200 ml in a 400-ml beaker. Then 2 to 3 g of sodium chloride and 10 ml of 10 per cent. hydrochloric acid were added and the solution was then raised to the boil. An excess (10 ml) of 10 per cent. phosphotungstic acid solution was then added, and the solution was boiled gently until the precipitate coagulated. This usually occurred within 5 minutes. The solution was cooled to room temperature and the precipitate was collected on a weighed No. 3 sintered-porcelain crucible. The precipitate was washed with about 200 ml of water and dried at 105° C to constant weight. A series of results obtained by this method is shown in Table I.

TABLE I

PRECIPITATION OF QUATERNARY SALTS AS PHOSPHOTUNGSTATES

Quaternary ammonium compound	Weight of precipitate, mg	Weight of precipitate calculated* [$Q_3PO_4.12WO_3$], mg	Average deviation, %
Cetyltrimethylammonium bromide	246·8 248·0	248·8	0·56
Myristyltrimethylammonium bromide	242·6 243·3	243·1	0·16
Lauryltrimethylammonium bromide	235·5 236·7	237·5	0·59
Decyltrimethylammonium bromide	228·7 230·0	231·9	1·08
Cetylpyridinium chloride	251·8 252·3	252·8	0·32
Myristylpyridinium chloride	246·6 247·2	247·1	0·12
Laurylpyridinium chloride	242·0 243·1	241·5	0·45
Decylpyridinium bromide	233·8 236·4	235·9	0·55
Cetylbenzylmorpholinium chloride	270·4 271·3	272·4	0·59
Cetylmethyldiethanolammonium chloride ..	260·1 261·7	260·8	0·31
p-tert.-Octylphenoxyethoxyethyldimethylbenzylammonium chloride	275·0 275·5	274·4	0·33

* Assuming solutions employed were exactly 0·01 *N*.

TABLE II

LOSS OF WEIGHT ON IGNITION OF QUATERNARY PHOSPHOTUNGSTATES

	1	2	3	4	5	6
	Weight of precipitate			Weight of residue		
Quaternary ammonium compound	found after heating at 105° C, mg	calculated,* mg	Deviation,† %	found after ignition, mg	calculated,‡ mg	Deviation,§ %
Myristyltrimethylammonium chloride ..	485·5	486·2	0·14	382·2	381·1	0·26
	484·4		0·37	380·6	380·4	0·05
Decyltrimethylammonium bromide ..	461·2	463·8	0·56	380·4	379·7	0·18
	462·4		0·30	381·2	380·6	0·16
Cetylpyridinium chloride	507·3	505·6	0·34	381·2	384·0	0·74
	504·6		0·20	379·7	381·0	0·34
Cetylbenzylmorpholinium chloride ..	543·0	544·8	0·33	380·8	380·5	0·08
	547·1		0·42	381·4	383·4	0·53
p-tert.-Octylphenoxyethoxyethyldimethylbenzylammonium chloride	545·8	548·5	0·49	378·4	380·0	0·42
	548·0		0·09	379·5	381·4	0·50

* Assuming solutions 0·01 *N* exactly.
† Deviation calculated from columns 1 and 2.
‡ Weight of residue calculated from column 1, the reaction $Q_3PO_4.12WO_3 \rightarrow HPO_3.12WO_3$ being assumed.
§ Deviation calculated from columns 4 and 5.

Experiments were then conducted to determine the loss on ignition of a selection of quaternary phosphotungstates. The various phosphotungstates were precipitated as before, but 40-ml samples of 0·01 *N* quaternary salt solutions were taken and the precipitates were collected on No. 3 sintered-porcelain crucibles. After being washed and dried at 105° C, the precipitates were ignited at a dull-red heat. The results obtained are shown in Table II.

TABLE III

MOLECULAR WEIGHTS CALCULATED FROM TABLE II

Quaternary ammonium compound	Molecular weight determined	Molecular weight calculated	Deviation, %
Myristyltrimethylammonium chloride	289·6	291·9	0·82
Decyltrimethylammonium bromide	277·8	280·2	0·86
Cetylpyridinium chloride	345·0	340·0	1·47
Cetylbenzylmorpholinium chloride	440·9	438·1	0·64
p-tert.-Octylphenoxyethoxyethyldimethylbenzylammonium chloride	453·5	448·1	1·24

DISCUSSION

The results given in Table I for a variety of surface-active quaternary ammonium compounds and the results obtained by Lematte *et al.* for some low molecular weight quaternary ammonium compounds show that precipitation as phosphotungstates is an accurate method of general application to quaternary ammonium bases.

The decomposition reaction *A* (p. 100) is confirmed by the results shown in Table II. This reaction is of great importance, as it permits the equivalent weight of the quaternary cation to be calculated. The anion present can be determined by conventional methods and hence the mean molecular weight of the quaternary ammonium compound can be determined.

The results given in Table II have been employed to calculate the molecular weights of the respective compounds in Table III.

In all other procedures that have been advocated for the determination of quaternary ammonium compounds a molecular weight must be assumed before the results can be calculated. By weighing the phosphotungstate after it has been dried at 105° C and again after ignition, the amount of quaternary ammonium compound present in the sample taken can be determined without knowledge of the molecular weight, which may be calculated from the same results if required. This is invaluable in the analysis of unknown samples, when even the most comprehensive qualitative analysis cannot establish the molecular weight of the quaternary ammonium compound present, and in addition most commercially available surface-active quaternary ammonium compounds are mixtures containing smaller or greater amounts of homologues of the main constituent.

The main disadvantage of this method is that ammonia and amines, *e.g.*, pyridine and trimethylamine, form insoluble phosphotungstates and these materials must therefore either be removed before the quaternary ammonium phosphotungstate is precipitated, or determined by some other means such as potentiometric titration and a correction for ammonium or amine phosphotungstate applied.

If the amine is volatile and the quaternary base is not decomposed on boiling, the amine may be removed by making the aqueous solution of the sample alkaline and boiling. The solution is then acidified with hydrochloric acid and the quaternary ammonium phosphotungstate precipitated in the usual manner.

A preferred method, which has been applied to a number of surface-active quaternary ammonium compounds, depends upon selective extraction. It has been found that the distribution coefficient of a surface-active quaternary ammonium compound between *iso*amyl alcohol and an aqueous solution of an inorganic acid is usually over 2000. (If the *iso*amyl alcohol - water - quaternary ammonium compound system is free from inorganic solute, separation into phases often does not take place.) Thus a solution of quaternary ammonium compound in *iso*amyl alcohol (0·1 to 0·5 *N*) may be freed from amine by washing with *N* hydrochloric acid. If the *iso*amyl alcohol solution is washed three times with an equal volume of aqueous acid, the loss of quaternary ammonium compound by transference to the aqueous phase is usually less than 0·2 per cent. The actual amount so transferred can be determined by combining the wash liquors and assaying by the bromophenol blue method of Barr, Oliver and Stubbings.[11] The *iso*amyl alcohol solution is then diluted with water, a little *iso*propanol or denaturant-free ethanol being added if necessary to give a clear solution, which is then treated with phosphotungstic acid in the usual way.

METHOD

It is assumed that the sample is free of interfering amines, *i.e.*, that if the initial sample contained amines these have been removed by one or other of the procedures discussed above.

REAGENTS—

Phosphotungstic acid—A 10 per cent. solution of $P_2O_5.24WO_3.xH_2O$.
Hydrochloric acid, 10 per cent.
Sodium chloride.

PROCEDURE—

Place a sample, W g, containing quaternary ammonium compound equivalent to about 0·0002 to 0·0006 gram-molecules in a 400-ml beaker and make up to about 250 ml with water. Make the solution slightly acid with hydrochloric acid, and then add an excess (10 ml) of 10 per cent. hydrochloric acid. Add about 2 g of sodium chloride and raise the solution to the boil. Add 10 ml of phosphotungstic acid solution and boil the mixture for 2 to 3 minutes, *i.e.*, until the precipitate has coagulated. Test the supernatant solution for completeness of precipitation by addition of a few drops of phosphotungstic acid. Cool the mixture to room temperature, collect the precipitate on a weighed No. 3 sintered-porcelain crucible and wash it with 100 to 200 ml of water. Dry the crucible at 105° C to constant weight and then ignite it at dull-red heat and reweigh it.

If the molecular weight only of the quaternary ammonium compound is to be determined, the phosphotungstate may be precipitated from a much larger (unweighed) sample, and this precipitate collected and washed on a Buchner funnel. After being dried at 105° C, a 1 to 2-g sample of the quaternary phosphotungstate should be weighed in an ordinary porcelain or silica crucible, and reweighed after being ignited at a dull-red heat. The accuracy of the molecular-weight determination is thus increased.

CALCULATION—

The reactions that have taken place are—

$$3QX + H_3PO_4.12WO_3 \rightarrow Q_3PO_4.12WO_3 \text{ (precipitate dried at 105° C)}$$
$$\downarrow$$
$$HPO_3.12WO_3 \text{ (residue left on ignition)}$$

(*i*) If the molecular weight of the quaternary ammonium compound is known, then—

$$\frac{100\,W_1M}{W(M-X+959{\cdot}3)} = \text{quaternary ammonium compound, per cent.} \quad .. \quad .. \quad (1)$$

where M = molecular weight of quaternary ammonium compound,
X = ionic weight of anion,
W = weight of sample, and
W_1 = weight of precipitate dried at 105° C.

(*ii*) If the molecular weight of the quaternary ammonium compound is unknown, then—

$$M = \frac{954{\cdot}3\,(W_1 - W_2)}{W_2} - 5 + X \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where M, X and W_1 are as in (*i*) and

W_2 = weight of residue left on ignition.

(*iii*) If the molecular weight of the quaternary ammonium compound is unknown, and it is required to calculate the percentage present in the sample without intermediate determination or calculation of the molecular weight, then by combining equations (1) and (2) the following equation is obtained—

$$\frac{100}{W}\left[W_1 - W_2\left\{1 - \frac{X-5}{954{\cdot}3}\right\}\right] = \text{quaternary ammonium compound, per cent.}$$

As only a few anions are normally present in quaternary ammonium compounds, the last term in the above equation can be reduced to a constant by substituting the appropriate value of X to give—

$$\frac{100}{W}[W_1 - KW_2] = \text{quaternary ammonium compound, per cent.}$$

where $K = 0{\cdot}9681$ for quaternary ammonium chlorides,
$K = 0{\cdot}9215$ for quaternary ammonium bromides, and
$K = 0{\cdot}8722$ for quaternary ammonium iodides.

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The Simultaneous Photometric Determination of Molybdenum and Tungsten in Silicate Rocks

By P. G. JEFFERY

A procedure is described for the simultaneous determination of molybdenum and tungsten in silicate rocks. Fusion with sodium hydroxide under oxidising conditions is used to extract these elements as molybdate and tungstate, respectively. Silica is removed by evaporation of the alkaline solution with hydrochloric acid. The complexes of molybdenum and tungsten with α-benzoinoxime are then extracted from the acid solution. When these elements are present in similar proportions, the complexes with toluene-3:4-dithiol are formed, and are extracted together, the molybdenum and tungsten being determined simultaneously by photometric measurement at wavelengths of 630 mμ and 680 mμ. If the proportion of either of these elements greatly exceeds that of the other, then the Allen and Hamilton procedure is applied for the separation of molybdenum from tungsten.

The conditions for the quantitative formation and extraction of the complexes of molybdenum and tungsten with toluene-3:4-dithiol were established by Allen and Hamilton,[1] who described a method for the determination of these elements in biological materials. These workers recorded the existence of absorption maxima at wavelengths of 630 mμ for the molybdenum complex with toluene-3:4-dithiol, and at 680 mμ for the tungsten complex. Provided that Beer's law is valid for the solutions used, and that there is no interaction between the two complexes when present together, then the optical density of a solution containing both metal complexes is given by—

$$\text{Log } I_0/I = t\,(E_1C_1 + E_2C_2),$$

where t is the length of the light path; E_1 and E_2 are the specific optical densities; and C_1 and C_2 are the concentrations of the component species. Measurement of the optical

densities of solutions containing both molybdenum and tungsten complexes with toluene-3:4-dithiol at 630 mμ and at 680 mμ permit the concentrations C_1 and C_2 to be calculated from the equations—

$$D_{630m\mu} = a\ [MoO_3] + b\ [WO_3]$$
$$D_{680m\mu} = a'\ [MoO_3] + b'\ [WO_3]$$

The values of the constants a and b at 630 mμ and at 680 mμ are determined experimentally, standard molybdenum and tungsten solutions being used.

The complexes of molybdenum and tungsten with toluene-3:4-dithiol are not, however, formed under the same conditions, a sulphuric acid concentration of 6 to 14 *N* being required for the quantitative formation of the molybdenum complex. At this acid concentration the corresponding tungsten complex is not formed. For the quantitative formation of the tungsten complex with toluene-3:4-dithiol, a pH of 0·5 to 2 is required. Between these pH values the formation of the molybdenum complex is not quite complete (Allen and Hamilton report "greater than 90 per cent."). In order to effect complete extraction of both molybdenum and tungsten, the following procedure is adopted.

First, the complex of tungsten with toluene-3:4-dithiol is developed at a pH of 2 and a temperature of 97° C, together with the major portion of the corresponding molybdenum complex. The remaining portion of the molybdenum is then converted into the complex with toluene-3:4-dithiol by adding diluted sulphuric acid (1 + 1 v/v) to bring the acid concentration to approximately 10 *N*, and then a further quantity of the organic reagent solution. Complete formation of both complexes is thus obtained in the same solution, from which extraction with light petroleum will quantitatively remove both the molybdenum and the tungsten.

In order to apply this procedure to the determination of molybdenum and tungsten, these elements are separated from the solution of the silicate rock, after removal of silica, by extraction with an organic solvent. The procedure of Allen and Hamilton[1] for the extraction of these elements as complexes with cupferron into *iso*amyl alcohol may be used for this purpose. An alternative procedure, which may be employed for the extraction of molybdenum and tungsten, and which also effects a separation from all interfering ions, is extraction as the complex with α-benzoinoxime from an acid solution with chloroform.

None of the remaining elements accompanying molybdenum and tungsten in the filtrate from an alkaline fusion interferes with the determination, with the exception of silica, which, on precipitation, removes a portion of both the molybdenum and the tungsten from the solution. This portion is recovered by dehydration of the silica with hydrochloric acid, evaporation with hydrofluoric acid, and fusion of the residue remaining after this treatment with a few milligrams of alkali carbonate. Chromium and vanadium, which, according to Knowles,[2] also form insoluble precipitates with α-benzoinoxime, are not extracted from acid solutions under the conditions employed, nor does their presence in the solution interfere with the extraction of molybdenum and tungsten.

Aluminium, iron, titanium, fluorine and phosphate, all of which may be present in the alkaline solution, are not extracted by the procedure described, nor do they interfere with the extraction of molybdenum and tungsten.

Fusion of the finely ground silicate rock with sodium or potassium hydroxide and nitrate in a nickel crucible, in the manner described by Harwood[3] for the determination of chromium and vanadium, is used to decompose the sample, and at the same time to effect a separation of molybdenum and tungsten from iron, manganese and other interfering metals. This method of decomposition is more rapid than fusion with alkali carbonate of a large (5-g) sample, although the method of Bennett and Pickup,[4] in which a platinum dish in an electric furnace at 1000° C is used, may also be used for this purpose. Fusion with alkali carbonate has no advantage over fusion with alkali hydroxide, as platinum dishes are required, and the time spent in extracting the solidified melt with water is, in general, much greater.

Experimental

The results obtained from the analyses of mixtures of standard molybdenum and tungsten solutions by the simultaneous photometric method described below are given in Table I. These results indicate that, at the concentrations employed, Beer's law is valid, and that there is no interaction between the two complexes when formed and extracted from the same solution.

TABLE I

THE RECOVERY OF MOLYBDENUM AND TUNGSTEN FROM MIXED STANDARD MOLYBDATE AND TUNGSTATE SOLUTIONS

Molybdenum trioxide added, μg	Tungsten trioxide added, μg	Molybdenum trioxide recovered, μg	Tungsten trioxide recovered, μg
10	10	9·8	10·1
10	20	9·8	20·2
20	20	19·9	20·1
10	30	10·0	30·0
20	30	20·1	29·6
30	30	30·2	29·3

The acid concentration for extraction of the complexes with α-benzoinoxime is not critical, both molybdenum and tungsten being completely recovered by four extractions from 0 to 1·8 *N* hydrochloric acid solutions, as shown in Fig. 1. Molybdenum is extracted

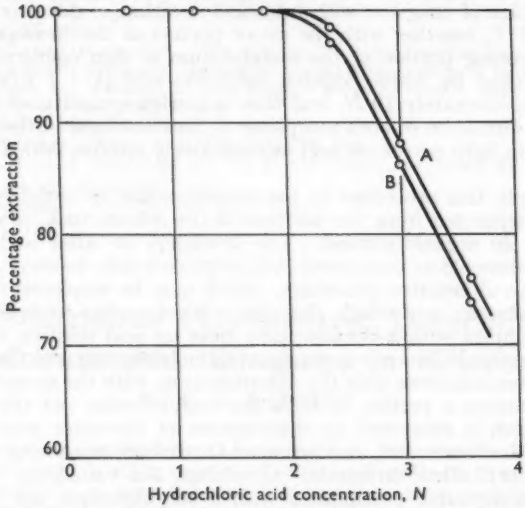

Fig. 1. Relation between percentage extraction and acid concentration: curve A, 20 μg of molybdenum trioxide extracted with α-benzoinoxime and chloroform; curve B, 20 μg of tungsten trioxide extracted with α-benzoinoxime and chloroform

more readily than tungsten; three extractions were found to be sufficient to transfer 20 μg of molybdenum to the organic solvent. As shown in Fig. 2, four extractions were found to be necessary to transfer a similar quantity of tungsten to the organic phase. Four extractions at a hydrochloric acid concentration of 1·5 *N* were used subsequently for all extractions of molybdenum and tungsten from silicate rocks.

TABLE II

LOSS OF MOLYBDENUM AND TUNGSTEN OWING TO CO-PRECIPITATION WITH SILICA

Sample	Molybdenum and tungsten not recovered from precipitated silica		Molybdenum and tungsten recovered from precipitated silica	
	MoO_3, μg	WO_3, μg	MoO_3, μg	WO_3, μg
1 g of granite	0·5	2·5	1·1	3·2
1 g of granite + 10 μg of molybdenum and 10 μg of tungsten	9·7	11·3	11·1	13·2
1 g of granite + 15 μg of molybdenum and 20 μg of tungsten	14·2	18·8	16·3	23·5

The loss of molybdenum and tungsten by co-precipitation with silica is shown by the results in Table II. These were obtained by adding aliquots of standard molybdate and tungstate solutions to the rock solution after fusion, and before filtration to remove the iron oxides, and so on.

Method

Reagents—

Water—The formation of a black toluene-3 : 4-dithiol complex, insoluble in light petroleum, during the course of the analysis was found to be due to the presence of copper in the water, derived from a copper still used for the distillation of water. Only water that had percolated through an ion-exchange demineraliser was subsequently used. Biodeminrolit mixed-bed resin was used for this purpose.[5]

Toluene-3 : 4-dithiol—Dissolve 1 g of the reagent in 300 ml of a 1 per cent. aqueous sodium hydroxide solution. When solution is complete, add 5 ml of thioglycollic acid. Store this solution in a refrigerator.

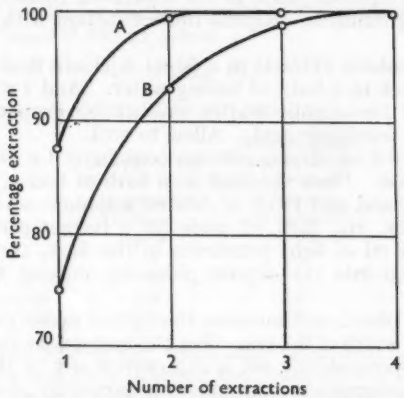

Fig. 2. Relation between percentage extraction and number of extractions: curve A, 20 μg of molybdenum trioxide extracted with chloroform and α-benzoinoxime from *N* hydrochloric acid; curve B, 20 μg of tungsten trioxide extracted with chloroform and α-benzoinoxime from *N* hydrochloric acid

Iron solution—Dissolve 1 g of pure iron wire in 20 ml of diluted sulphuric acid (1 + 1 v/v), and dilute to 1 litre.

Light petroleum—The product X3B, supplied by the Shell Company of East Africa, with a nominal boiling range of 100° to 132° C, was used. Purify this solvent before use by shaking with concentrated sulphuric acid, neutralising, and washing with water.

Sulphuric acid, diluted (1 + 1 v/v)—Mix equal volumes of water and concentrated sulphuric acid.

Sulphuric acid, diluted (1 + 3 v/v)—Mix equal volumes of water and diluted sulphuric acid (1 + 1 v/v).

α-Benzoinoxime—Dissolve 2 g of reagent in 100 ml of ethanol.

Standard molybdenum and tungsten solutions—Dissolve 0·25 g of pure molybdenum oxide in a small quantity of ammonium hydroxide, and dilute to 250 ml with water. From this solution, prepare a working solution containing 10 μg per ml by dilution with water when required. Prepare the standard tungsten solution similarly, using 0·25 g of tungstic oxide.

Procedure—

Fuse approximately 25 g of sodium hydroxide with 1 g of sodium nitrate in a nickel crucible until all water is expelled. When cold, transfer approximately 5 g of the silicate rock to the surface of the melt, re-melt the contents of the crucible, and maintain in a molten condition for 1 hour. Extract the cold melt with hot water containing a few drops of

ethanol. Collect the residue on a large hardened filter-paper, and wash the precipitate well with a hot 2 per cent. sodium carbonate solution. Discard this residue. Evaporate the filtrate to dryness with concentrated hydrochloric acid in a platinum dish to precipitate and dehydrate silica. Add 20 ml of concentrated hydrochloric acid and approximately 200 ml of water. When all soluble salts have passed into solution, collect the precipitated silica on an open-textured filter-paper, and wash the precipitate well with hot water. Reserve the filtrate. Ignite the precipitate in a platinum crucible, and remove the silica by evaporation with hydrofluoric acid in the usual way; avoid overheating the residue. Recover the traces of molybdenum and tungsten by fusion of this residue with a few milligrams of fusion mixture, and extraction with water. Add this solution to the filtrate from the silica precipitation.

Transfer this solution quantitatively to a large separating funnel, and dilute to 250 ml. Add 2 ml of α-benzoinoxime solution, and shake well. Add 10 ml of chloroform, and shake for a period of 1½ minutes to extract the molybdenum and tungsten into the organic phase. Allow the two phases to separate, and carefully withdraw the lower organic layer. Add 5 ml of chloroform to the aqueous phase in the separating funnel, and repeat the extraction for a further period of 1½ minutes. Repeat this extraction with two further 5-ml portions of chloroform.

Collect the four chloroform extracts in a 50-ml Kjeldahl flask, and distil off the organic solvent by placing the flask in a bath of boiling water. Add 1 ml of diluted sulphuric acid (1 + 3 v/v), and destroy the organic matter present by evaporation to fumes, first with nitric acid and then with perchloric acid. Allow to cool.

Add 20 ml of water, 0·5 ml of iron solution containing 1 g of iron per litre and 5 ml of toluene-3:4-dithiol solution. Place the flask in a bath of boiling water. After a period of 1 hour, remove the flask, and add 12 ml of diluted sulphuric acid (1 + 1 v/v) and 3 ml of toluene-3:4-dithiol solution, and then set aside for a further period of 1 hour. Cool, and by pipette put exactly 10 ml of light petroleum in the flask, cork it well, and extract the molybdenum and tungsten into the organic phase by shaking for three separate periods of 1½ minutes each.

Separate the organic phase, and measure the optical density at wavelengths of 630 mμ and 680 mμ, using a band width of 0·3 mμ. For the instrument that I used (Uvispek, Hilger & Watts Ltd.) this corresponded to a slit width of 0·06 mm at the wavelengths employed.

When one element predominates, the following procedure, essentially the same as that described by Piper and Beckwith[6] and Allen and Hamilton,[1] may be employed to separate molybdenum from tungsten.

Proceed as described above to obtain the solution of molybdenum and tungsten in sulphuric acid, all the organic matter having been removed by evaporation to fumes of sulphuric acid with nitric and perchloric acids. Add 10 ml of diluted sulphuric acid (1 + 1 v/v), 7 ml of water and 1 ml of toluene-3:4-dithiol solution. Set this solution aside at room temperature for 1 hour. Add 10 ml of light petroleum, and extract the molybdenum-toluene-3:4-dithiol complex by shaking for three separate periods of 1½ minutes as described above. Measure the optical density of the organic phase at 630 mμ.

Transfer the aqueous phase remaining after the extraction of molybdenum, or an aliquot of it containing not more than 40 g of WO_3, to a 50-ml Kjeldahl flask, and evaporate the solution to approximately 0·5 ml. Neutralise the remaining sulphuric acid with dilute ammonium hydroxide (2 *N*), and boil off the excess of ammonia. Add 1 ml of diluted sulphuric acid (1 + 3 v/v) and 20 ml of water. Add 3 ml of the toluene-3:4-dithiol solution, and place the flask on a boiling-water bath for a period of 1 hour. Cool the flask and its contents to room temperature, and add 10 ml of light petroleum. Extract the tungsten complex into the organic phase by shaking for three separate periods of 1½ minutes each. Remove the organic layer, and measure the optical density at 680 mμ.

A determination of molybdenum and tungsten in the reagents should be made, and the blank thus obtained subtracted from the results obtained with the rock samples.

Discussion

Molybdenum and tungsten may be determined simultaneously in silicate rocks provided that the ratio of the contents of these metals does not exceed about 1 to 12. No difficulty is experienced in determining molybdenum and tungsten when they are not present below this ratio, although the separation of molybdenum is then necessary.

Results obtained on various samples by the method described in this paper and results obtained after adding known amounts of molybdenum and tungsten to them are shown in Table III.

TABLE III

RECOVERY OF MOLYBDENUM AND TUNGSTEN ADDED TO SILICATE ROCKS

Sample	Molybdenum trioxide found, μg	Molybdenum trioxide added, μg	Total molybdenum trioxide found, μg	Tungsten trioxide found, μg	Tungsten trioxide added, μg	Total tungsten trioxide found, μg
Granite, Singo	5·6	10	16·0	58·0	10	66·0
Mudstone, Karroo Beds, Entebbe..	13·8	5	19·0	10·5	5	15·5
Granite, Mubende	5·5	10	15·2	18·3	5	23·0

Some further results for silicate rocks found in the Uganda Protectorate are shown in Table IV, together with results for the same rocks by the methods of Kuroda and Sandell[7] for molybdenum and of Sandell[8] for tungsten.

TABLE IV

COMPARISON OF RESULTS

Sample	Molybdenum trioxide found by present method, p.p.m.	Molybdenum trioxide found by method of Kuroda and Sandell,[7] p.p.m.	Tungsten trioxide found by present method, p.p.m.	Tungsten trioxide found by method of Sandell,[8] p.p.m.
Granite, Mubende	1·1	1·2	3·7	3·5
Granite, Entebbe	1·0	1·3	8·9	8·8
Mudstone, Karroo Beds, Entebbe..	2·8	3·1	2·1	2·2
Phonolite, Toror Hills, Karamoja ..	6·2	7·9	9·4	8·5

From these results it can be seen that remarkable agreement has been obtained, although there is a tendency for the results by Kuroda and Sandell's method for molybdenum to be higher than those by the present method. This can probably be ascribed to the intrinsic error of the thiocyanate method, in which the thiocyanate complexes of molybdenum and tungsten are extracted together and reported as molybdenum.

The author is grateful to the Acting Director, Geological Survey of Uganda, for permission to publish this paper.

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Determination of Strontium in Sea Water by Using both Radioactive and Stable Isotopes

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By means of the isotope-dilution technique with strontium-89, the strontium content of a sample of North Atlantic sea water was found to be 8·0 ± 0·1 mg per litre. By means of the radioactivation technique with strontium-89, the strontium content found was 8·0 mg per litre, whereas with strontium-87 it was 8·1 ± 0·3 mg per litre. When the isotope-dilution technique with strontium-84 and strontium-88 was used, the strontium content found was 8·1 ± 0·1 mg per litre.

THE results of nearly a dozen investigations of the strontium content of sea water have been published, the values ranging from 6 to 50 mg per litre; the generally accepted value until 1951 was 13 mg per litre. The most recent determinations have been those of Smales,[1] Odum[2] and Chow and Thompson[3] (*q.v.* for further references). Smales employed a combination of radiochemistry and flame photometry, and obtained a tentative value of approximately 10 mg per litre, whereas Odum and Chow and Thompson used flame photometry and obtained 8·1 and 8·2 mg per litre, respectively. The present work was undertaken to provide a check by independent methods of the latest reported values, which, when this work was started, were those of Smales and of Odum.

Experimental

Isotope-dilution technique with strontium-89—

To either 1 or 2 litres of sea water (from a depth of 100 metres at 43° 00′ N, 17° 34′ W) was added a 5 or 10-ml aliquot of $^{89}SrCl_2$ solution containing 2·00 mg of total strontium per ml and with an initial activity of about 400 counts per minute per ml as measured on the top shelf of an end-window Geiger - Müller counter assembly. Sufficient saturated sodium carbonate solution was added to precipitate the carbonates, which were separated by centrifuging, washed three times with demineralised water and dissolved in the minimum volume of 6 *M* nitric acid. The solution was evaporated to dryness, and the residue was taken up in the minimum volume of water required to dissolve the precipitated salts, the calcium and strontium being separated by treatment with fuming nitric acid and then with absolute ethanol. To each 10 ml of solution were added 26 ml of fuming nitric acid to precipitate strontium nitrate, most of the calcium being left in solution. After the mixture had been cooled in an ice-bath and then centrifuged, the nitric acid was decanted and 10 ml of absolute ethanol were added slowly to dissolve calcium nitrate preferentially. After the solution had been centrifuged, the strontium nitrate was dissolved in 5 ml of demineralised water and boiled to remove ethanol. Two more extractions with fuming nitric acid and absolute ethanol were followed by precipitation of strontium sulphate with ammonium sulphate solution from the final, hot strontium nitrate solution. After the strontium sulphate had been washed three times with water or a dilute ethanol solution and then once with absolute ethanol, the material was transferred as an absolute ethanol slurry to a weighed aluminium counting tray and dried under an infra-red lamp. It was then weighed and counted and the activity was compared with that of a measured weight of strontium sulphate precipitated from the original $^{89}SrCl_2$ solution. Between 25,000 and 50,000 counts were registered with each precipitate and were corrected for coincidence, background and a combination of self-absorption and scattering.

The correction factors for self-absorption and scattering were determined by precipitating about 500 mg of strontium sulphate (containing strontium-89) and slurrying from 4 to 150-mg portions on to aluminium trays of closely similar weights. After counting and making the necessary corrections, a plot of the specific activities (counts per minute per milligram) against weight of precipitate gave a curve with a broad maximum at about 100 mg (47 mg per sq. cm). Division of each specific activity into the specific activity of 100 mg gave factors that, when plotted (linear scale) against the weight of strontium sulphate (log. scale), gave an essentially straight line between 10 and 80 mg of strontium sulphate. The activity of

any sample precipitate may then be related to what it would be at the weight of the standard strontium sulphate precipitate.

After the above corrections had been made to the observed counts, the strontium content of the sea water was calculated by using an equation expressing the fact that the total strontium-89 activity was the same in both the aliquot of solution added to the sea water and in its mixture with the sea water, *i.e.*—

$$S_m . m = S_x (m + x)$$

where S_m = specific activity of standard strontium-89 solution, in counts per minute per mg of strontium sulphate,
m = weight of strontium in the aliquot taken,
S_x = specific activity of strontium sulphate from sea-water mixture, and
x = weight of strontium in the volume of sea water taken.

The results of four experiments are given in Table I.

TABLE I

STRONTIUM CONTENT OF SEA WATER BY ISOTOPE DILUTION WITH STRONTIUM-89

Volume of sea water, litres	Strontium added as ^{89}Sr solution, mg	Strontium in sea water, mg per litre
1	20	7·96
2	20	8·15
2	10	8·05
2	10	7·99

Mean and standard deviation, 8·0 ± 0·1.

RADIOACTIVATION METHOD (VIA STRONTIUM-87)—

In this method, 1 or 2-ml volumes of sea water and of standard solution (26·6 mg of strontium per litre), sealed in silica tubes, were irradiated simultaneously in the pile for times varying between 5 and 7 minutes. Immediately after irradiation the contents of the tubes were washed out for chemical treatment.

After 10 ml of strontium chloride solution (4 mg of strontium per ml) had been added, strontium and other insoluble carbonates were precipitated by adding 5 ml of sodium carbonate solution (200 mg of anhydrous sodium carbonate per ml). The precipitate was centrifuged and washed three times with water and then dissolved in 3 or 4 drops of concentrated hydrochloric acid. A "scavenge" with ferric hydroxide, ammonia solution being used, was followed by two successive treatments with fuming nitric acid and absolute ethanol, as described above. Before the first treatment with fuming nitric acid about 2·5 mg of calcium ions were added as hold-back carrier. A second iron scavenge was followed by a barium chromate precipitation in which 1 ml of barium chloride solution (5 mg per ml) and 3 ml of acetate buffer solution (2 ml of 6 *M* ammonium acetate plus 1 ml of 6 *M* acetic acid) were added, the solution was heated in a hot-water bath, and the barium was precipitated with 0·5 ml of 1·5 *M* sodium chromate solution. After the filtrate had been collected in a clean centrifuge tube, it was reheated and 4 ml of 2 *M* ammonium sulphate were added all at once and the mixture was then digested for 10 to 15 minutes to give a good crystalline precipitate. This was washed three times with 25 per cent. aqueous ethanol and once with absolute ethanol and was then transferred to a weighed aluminium tray as an ethanol slurry, dried under an infra-red lamp, weighed and counted by using a methane-flow proportional counter. The observed count was corrected for coincidence when necessary, for background and for chemical yield.

The standard solution and sea water were assumed to have the same specific activity due to strontium-87, so that—

$$\frac{\text{weight of strontium in standard}}{\text{corrected count rate of standard}} = \frac{\text{weight of strontium in sea water}}{\text{corrected count rate of sea water}},$$

and hence the weight of strontium per unit volume of sea water could be calculated.

Radiochemical purity was checked by following the decay of each sample for 2 to 4 hours, and all were satisfactory in this respect, with half-lives of about 2·8 hours.

The results are given in Table II.

RADIOACTIVATION METHOD (VIA STRONTIUM-89)—

This method involved the neutron irradiation in the pile of relatively large volumes of liquids. For this purpose the apparatus designed by Pate[4] was used, in which eight, open, narrow-necked silica tubes, of 8 to 10-ml volumes, held vertically in a graphite housing placed inside an aluminium can, could be simultaneously irradiated.

After being weighed, four of the tubes were half-filled with standard strontium chloride solution (26·6 mg of strontium per litre) and the remaining four with sea water. The tubes were then reweighed. After 2-weeks' irradiation in a thermal column of the pile and 1 week out of the pile, to permit unwanted activities to decay to safe levels, the contents of the tubes were washed out for chemical treatment identical to that outlined immediately above, except that no calcium hold-back carrier was added.

Radiochemical purity was checked by plotting an aluminium-absorption curve. A Feather analysis (UX standard) gave a maximum beta energy of 1·58 MeV, in excellent agreement with results obtained on neutron irradiation of Specpure strontium carbonate.

An end-window Geiger - Müller counter was used for counting strontium-89.

The result of the single experiment is given in Table II.

TABLE II

STRONTIUM CONTENT OF SEA WATER BY RADIOACTIVATION

Strontium isotope used	89	87	87	87	87	87
Strontium in sea water, mg per litre	8·05	7·92	8·20	8·46	7·93	7·79

Mean and standard deviation, 8·1 ± 0·3.

ISOTOPE-DILUTION TECHNIQUE WITH STRONTIUM-84—

In this method, aliquots of a strontium chloride solution of known strontium content, containing 50·7 per cent. of strontium-84 and 32·3 per cent. of strontium-88 (*i.e.*, enriched in strontium-84 by electromagnetic separation) were added to 2-litre portions of sea water and the carbonates were precipitated with sodium carbonate solution. The precipitate was separated by centrifuging, washed three times with water and then dissolved with nitric acid; the solution was evaporated nearly to dryness, and the residue was taken up with the minimum volume of water and treated three times each with fuming nitric acid and absolute ethanol as described above. The final aqueous solution of strontium nitrate was acidified with hydrochloric acid, 1 ml of saturated oxalic acid was added, and strontium oxalate was precipitated from the hot solution by adding several drops of 6 *M* ammonium hydroxide. After being washed several times with water the precipitate was transferred to an aluminium dish and dried under an infra-red lamp, when it was ready for analysis with a solid-source mass spectrometer.

Mass-spectral analysis gave a value R for the ratio of the abundances of the isotopes of masses 88 and 84. The abundance of strontium-88 was composed of that due to the sea water plus that added in the "spike," and similarly for strontium-84, *i.e.*—

$$R = \frac{A_N^{88} X + A_S^{88} W}{A_N^{84} X + A_S^{84} W},$$

where A_N^{88} is the natural abundance of strontium-88 (82·7 per cent.),
A_S^{88} is the abundance of strontium-88 in the spike (32·3 per cent.),
A_N^{84} is the natural abundance of strontium-84 (0·55 per cent.),
A_S^{84} is the abundance of strontium-84 in the spike (50·7 per cent.),
W is the weight of strontium in the spike, and
X is the weight of strontium in the volume of sea water taken.

The value of X is then readily calculated.

The results are given in Table III.

CONCLUSIONS

The results agree closely with those obtained recently by flame-photometric methods, although they are not so precise because of the techniques employed. The strontium content of North Atlantic sea water is evidently about 8·1 mg per litre as determined by our methods. Inherent lack of precision precludes relating this value to a definite chlorinity.

TABLE III

STRONTIUM CONTENT OF SEA WATER BY ISOTOPE DILUTION WITH STRONTIUM-84

Volume of strontium-84 solution (0·210 mg of Sr per ml), ml	Isotope ratio, R	Strontium in sea water, mg per litre
5	22·4 ± 0·3	8·23
5	21·8 ± 0·1	7·97
10	12·3 ± 0·15	8·20
20	6·62 ± 0·08	8·05
	Mean and standard deviation, 8·11 ± 0·12	

We are grateful to Mr. K. L. Aitken, for carrying out the mass-spectrometric analyses.

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October 20th, 1955

The Determination of Dissolved Oxygen in Water Containing Reducing Substances

BY I. DAVIES, M. N. REDFEARN AND D. E. Y. REMER

The effect of interfering substances on the Winkler method for determining dissolved oxygen in water is shown experimentally. A system of ion-exchange columns is proposed in which the interference is removed while the sample is being taken, thus enabling the Winkler method to be applied without modification.

THE basis of most of the modern methods for the determination of dissolved oxygen in water is that of Winkler.[1] The determination of minute traces of dissolved oxygen down to 0·001 p.p.m. is very important in power-station control, and the Winkler method, suitably modified by Hewson and Rees,[2] is in general use in this country and is capable of giving excellent results.

The Winkler test fails if the water contains interfering substances; the extent of this failure is shown in Table I. From this table it will be seen that whereas ferric and cupric ions are relatively harmless, ferrous, cuprous, sulphite and sulphide ions all give rise to an apparent loss of dissolved oxygen.

Many methods have been proposed to overcome this difficulty, but they all suffer from some disadvantage. The Rideal - Stewart modification[3] introduces additional reagents that may contain more dissolved oxygen than the sample; the azide modification[4] will not deal with many interferences, and that of Schwartz and Gurney[5] involves taking two blanks for each sample tested. The A.S.T.M. method,[6] besides involving a theoretical objection, has been found to give an appreciably less accurate result.[7]

It is well known that by means of a mixed-bed ion-exchange column it is possible to prepare water practically free of ionic impurities, and it was conceived that such a column might be used to purify the water being sampled for dissolved oxygen. The interferences may thus be removed and the simple Winkler test applied without modification. While this paper was being prepared, Parkhouse[8] put forward this idea. Potter[9] pointed out that the excellent agreement reported by Parkhouse must have been fortuitous for reasons that we discuss below. We have however largely overcome these difficulties by using a combination of resin columns.

Experimental

Preliminary procedure—

A resin column containing a mixture of De-Acidite FF and Zeo-Karb 225 was prepared. The column was about 4 cm in diameter and 60 cm long. The column was fed from the bottom with feed water from a de-aerator. The water used was of good quality, its conductivity, out of contact with atmospheric carbon dioxide, usually ranging from 0·7 to 1·7 reciprocal megohms per cm cube at 20° C. Arrangements were made by which strong

Table I

Effect of some impurities on results by the Winkler test

Interfering ion	Amount of ion added, p.p.m.	Oxygen in sample, p.p.m.	Oxygen found, p.p.m.
$Fe^{\cdot\cdot\cdot}$	0·05	0·036	0·033
$Fe^{\cdot\cdot\cdot}$	0·15	0·057	0·061
$Fe^{\cdot\cdot\cdot}$	0·20	0·079	0·069
$Cu^{\cdot\cdot}$	0·05	0·049	0·053
$Cu^{\cdot\cdot}$	0·10	0·069	0·074
$Cu^{\cdot\cdot}$	0·25	0·081	0·089
$Fe^{\cdot\cdot}$	0·05	0·033	0·029
$Fe^{\cdot\cdot}$	0·10	0·050	0·040
$Fe^{\cdot\cdot}$	0·20	0·073	0·053
$Fe^{\cdot\cdot}$	1·36	0·016	0·001
$Cu^{\cdot}$	0·17	0·040	0·033
$Cu^{\cdot}$	0·35	0·057	0·037
$Cu^{\cdot}$	1·42	0·019	0·003
SO_3''	0·10	0·026	0·013
SO_3''	0·20	0·026	0·001
S''	0·10	0·029	− 0·029
S''	0·20	0·030	− 0·048

solutions of interfering ions could be injected into the feed water, and sampling points were provided for the raw feed water, the feed water and interfering solutions and the column outlet. The apparatus was fitted with flow meters on the raw water and interference injection lines.

The results obtained with this apparatus for ferrous and cuprous ions are shown in Table II.

Table II

Effect of a mixed-bed column on ferrous and cuprous interference

Interfering ion	Amount of ion present, p.p.m.	Dissolved oxygen in raw water, p.p.m.	Apparent dissolved oxygen in water fed to column, p.p.m.	Dissolved oxygen in column outlet, p.p.m.
	Nil	0·023	0·024	0·026
$Fe^{\cdot\cdot}$	4·70	0·023	− 0·006	0·024
	Nil	0·014	0·024	0·026
$Fe^{\cdot\cdot}$	6·50	0·014	− 0·001	0·030
	Nil	0·039	0·040	0·040
$Fe^{\cdot\cdot}$	4·90	0·039	− 0·004	0·026
	Nil	0·026	0·031	0·027
$Cu^{\cdot}$	3·07	0·016	0·003	0·027
$Cu^{\cdot}$	0·92	0·027	0·004	0·029

When the column was first used, it was found that the water gained in dissolved oxygen content on passing through it. This effect is probably due to oxygen occluded in the resin, as several hours running is sufficient to remove it. After the column has been shut off and even if stored under de-aerated water, a high dissolved-oxygen content is still found in the first few litres of water drawn through it. This soon passed off and reasonable agreement was soon found. It is evident that an equilibrium is set up between oxygen occluded on the resins and oxygen dissolved in the water. While the column is being used, this equilibrium is slowly established.

DISCUSSION—

At this stage of the work doubt was cast upon the applicability of the method by an observation of Stanesby[10] that came to our notice. This worker had loaded a column of anion-exchange resin by treatment with a strong sodium sulphite solution and, after rinsing the column, he found it reduced the dissolved oxygen content of the water passing through it. Thus a 2-cm diameter 20-cm column of Amberlite IRA-400(OH) in the sulphite form reduced the dissolved oxygen by about one-third.

This observation is in agreement with those published by Sansoni[11] and Cassidy,[12] who used different reducing-agent - resin combinations.

A check on our experimental column showed that the effect could not be obtained on the resins if they are treated with more-dilute sulphite solutions. For example, the results obtained with the demineralising column after passage of 120 ml of *N* sodium sulphite solution are given in Table III.

TABLE III

EFFECT ON DISSOLVED OXYGEN CONTENT OF WATER PASSED THROUGH A COLUMN LOADED WITH *N* SODIUM SULPHITE SOLUTION

Dissolved oxygen in inlet water, p.p.m. ..	0·010	0·021	0·022	0·020
Dissolved oxygen in outlet water, p.p.m. ..	0·011	0·016	0·018	0·021

A demineralising column in regular use may come into contact with many different types of impurity and by a combination of impurities may act as a de-aerator, even though it was never treated with strong solutions of reducing agents. It was therefore felt desirable to modify the method described above to deal with such eventualities.

MODIFIED PROCEDURE—

A column, 2·5 cm in diameter and 15 cm long, was filled with Amberlite IRA-400(OH) resin that had been converted to the (MnO_4) form by soaking in a 2 per cent. solution of potassium permanganate. The column was thoroughly washed free from permanganate. In accordance with Sansoni's proposed nomenclature, we term this an "oxite" column.

This column was found to oxidise completely dilute solutions of ferrous and sulphite solutions that were passed through it. We could not check the completion of cuprous oxidation owing to our inability to determine the $Cu^{..}$ - $Cu^{.}$ ratio at high dilution. A dilute sulphide solution produced a break up of the resin granules.

As ferric and cupric ions do not interfere with the Winkler test, an attempt was made to use an "oxite" column to remove interference without the use of a demineralising column. It was found, however, that such a column apparently added dissolved oxygen to the water that passed through it. Similar results were obtained on a column loaded with dichromate ions.

TABLE IV

THE APPARENT GAIN IN DISSOLVED OXYGEN IN WATER PASSED THROUGH "OXITE" COLUMNS

	Permanganate as oxidising agent			Dichromate as oxidising agent		
Dissolved oxygen in feed, p.p.m. ..	0·029	0·019	0·033	0·018	0·007	0·025
Dissolved oxygen in outlet, p.p.m. ..	0·057	0·036	0·068	0·054	0·018	0·035

The results shown in Table IV were obtained after the columns had been washed for at least 72 hours. It was thought that the oxidising ion was being gradually leached out of the resin and that this was affecting the Winkler test and causing the high values that were obtained. This was confirmed by placing the demineralising column after the "oxite" column. The results obtained by such a combination of columns in dealing with interference is shown in Table V. It will be noted that the agreement is fairly good; the method breaks down only in the presence of sulphide. This may be due to the oxidation by the "oxite" resin going to the stage of free sulphur, which will later pass unchanged through the mixed-bed column and interfere in the Winkler test.

TABLE V

THE EFFECT OF AN "OXITE" COLUMN FOLLOWED BY A MIXED-BED COLUMN

Dissolved oxygen in raw water, p.p.m.	Nature and amount of impurity, p.p.m.	Dissolved oxygen in mixed-bed column outlet, p.p.m.
0·039	11·0 of SO_3''	0·040
0·047	8·9 of SO_3''	0·067
0·041	15·2 of SO_3''	0·047
0·036	9·4 of SO_3''	0·039
0·056	8·2 of SO_3''; 0·80 of $Cu^{\cdot}$; 0·80 of $Fe^{\cdot\cdot}$	0·056
0·047	10·0 of SO_3''	0·054
0·048	11·3 of SO_3''; 0·89 of $Cu^{\cdot}$; 0·89 of $Fe^{\cdot\cdot}$	0·044
0·019	15·2 of SO_3''; 1·23 of $Cu^{\cdot}$; 1·23 of $Fe^{\cdot\cdot}$	0·021
0·029	14·2 of SO_3''; 1·13 of $Cu^{\cdot}$; 1·13 of $Fe^{\cdot\cdot}$	0·039
0·031	8·0 of SO_3''; 0·63 of $Cu^{\cdot}$; 0·63 of $Fe^{\cdot\cdot}$	0·043
0·024	6·7 of SO_3''; 0·54 of $Cu^{\cdot}$; 6·7 of $Fe^{\cdot\cdot}$	0·034
0·071	8·2 of S''	0·029
0·059	4·9 of S''	0·049
0·041	9·4 of S''	0·093
0·031	12·0 of S''	0·040

CONCLUSIONS

A method has been produced by which the Winkler test can be applied without modification to water containing traces of reducing substances. The method will not apply if much sulphide is present.

We consider this method to be capable of wider application and are making preliminary experiments on grossly polluted estuarine waters, which we hope to publish later.

Our thanks are due to Mr. H. V. Pugh, Controller, London Division, Central Electricity Authority, for permission to publish and to Mr. E. A. Howes, Chief Chemist, London Division, Central Electricity Authority, for valuable suggestions.

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BATTERSEA GENERATING STATION
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The Vitamin-A and Carotene Potency of National (Household) Butter Determined by a Chromatographic - Ultra-violet Spectrophotometric Method of Assay

By W. G. SCOTT and R. J. TAYLOR

The chromatographic method of analysis for margarine of Boldingh and Drost, as modified and subsequently adopted as the official method, has been applied to a series of imported and English butters, and their vitamin-A and carotene potencies have thereby been determined.

Numerous papers have appeared in the literature dealing with the vitamin-A and carotene potency of butter. They can be divided broadly into those about the method of assay and those about the effects of such variants as season, location and herd breed on the potency. The quantitative significance of the results in the second group would depend on the method of assay used. Adequate reference to both types of investigation can be found in papers by McDowell[1] and by McDowell and McDowall.[2]

In earlier work, potencies were determined either by a biological method, which does not distinguish between vitamin A and carotene, or by separate physico-chemical assays of the two compounds, that for vitamin A being usually some form of the Carr - Price test, in which corrections for the carotene - antimony trichloride reaction were necessary. Morton, Lord and Goodwin,[3] however, describe a method of phase separation leaving only xanthophyll with the vitamin A: this does not have a Carr - Price reaction comparable with that of vitamin A and no correction for it is required when this method of test is used. In later work, Goodwin and Morton[4] used, and evidently preferred, a simple chromatographic procedure based on defatted bone meal as a means of separating carotene (other than xanthophyll) and vitamin A.

It is recognised generally that the most accurate and reproducible technique of assaying both vitamin A and carotene is spectrophotometric; by measurements in the region of 325 mμ for vitamin A, and in the region of 450 mμ for carotene. The international units of activity have been defined as those of 0·344 μg of vitamin-A acetate and 0·6 μg of β-carotene, respectively, and, since the $E^{1\%}_{1cm}$ values of the pure compounds have been determined,* factors can be applied in any given instance to determine the vitamin-A and β-carotene potencies. Before such factors are used it is important that the spectrophotometric data should satisfy the criterion for purity. Attempts have been made to achieve this,[1] but so far the Carr - Price test has remained the preferred method for vitamin A. It has been found, however, that the method of Boldingh and Drost[5] for margarine, modified only in detail, is applicable directly to butter; and application of the method has permitted us to assay both vitamin A and carotene spectrophotometrically in one operation, since all carotenoids are isolated physically from vitamin A in the course of it.

The results given in this paper were obtained during the period October 1952 to March 1953 on samples of imported blended butters and of home produced butters supplied by the Ministry of Food.

The significance of the results lies not only in the establishment of mean levels of vitamin A and carotene in butter as offered for sale in Britain, but also in the degree of correlation attainable between two laboratories on a product of this type when a chromatographic - ultra-violet method of assay is used. The method in its final form was that adopted subsequently as the official method of assay for margarine, and reference may be made to the Statutory Instrument[6] for details.

The following example illustrates the method of analysis—

Composition of butter: 80 per cent. of New Zealand and 20 per cent. of Danish.

A 10-g sample was saponified, the unsaponifiable matter was dissolved in light petroleum and the solution was chromatographed.

* The values for vitamin A are known with reasonable certainty (*Nature*, 1951, **168**, 598). The same degree of certainty does not exist at present for β-carotene, and we have used a value of 2500 as the best approximation to the various published values.

Carotene fraction: this was eluted before the vitamin A. The volume of the eluate was adjusted to 20 ml in light petroleum (equivalent to a 50 per cent. solution of the original sample). The spectrophotometric results were—

Wavelength, mμ	440	442	444	446	448	450
Optical density (in 1-cm cuvette) ..	0·920	0·948	0·975	0·975	0·970	0·950

$E^{1\%}_{1cm}$ at 446 mμ = 0·0195.

The carotene fraction is free from xanthophyll, but contains some α-carotene. The amount will vary between samples, but to a first approximation the carotene fraction from the chromatogram can be taken as equivalent to 90 per cent. of β-carotene. If the latter has an $E^{1\%}_{1cm}$ value of 2500 in light petroleum, then the appropriate factor for converting the $E^{1\%}_{1cm}$ value of the chromatographic fraction to carotene potency is

$$\frac{90}{100} \times \frac{1{\cdot}67}{2500} \times 10^6 = 600.$$

Hence carotene potency of sample = 0·0195 × 600 = 11·7 i.u. per g.

Vitamin-A fraction: one-half of each aliquot reacting to antimony trichloride reagent was bulked and adjusted to 10 ml in light petroleum, to give the equivalent of a 50 per cent. solution of the original sample. The spectrophotometric results were—

Wavelength, mμ ..	270	280	290	309	322	324	326	334	340	350	360	370
Optical density (in a 1-cm cuvette) ..	0·147	0·198	0·281	0·525	0·600	0·605	0·600	0·524	0·430	0·260	0·137	0·057
Ratio				0·867		1·000		0·866				

NOTE—The "ratio" figures are relative optical densities at reference points on which the validity of the assay is based.

$E^{1\%}_{1cm}$ value at 324 mμ = 0·0121.
Vitamin-A potency = 0·0121 × 1830.
= 22·1 i.u. per g.

One-hundred and six samples of imported butters, and ten samples of English butters were examined. The imported butters included New Zealand, Australian and Danish, single or blended. Full details of the individual samples and the results obtained in the two laboratories are given in Table I: blanks in the Government laboratory list indicate samples on which assays were not made in that laboratory.

The average values were as follows—

Type of butter	Vitamin-A potency		Carotene potency	
	Government laboratory, i.u. per g	Unilever laboratory, i.u. per g	Government laboratory, i.u. per g	Unilever laboratory, i.u. per g
Imported	23·9	23·8	9·8	9·7
English	21·7	20·7	7·3	7·1
Over-all (weighted) average	23·7	23·5	9·5	9·5

The tests were made on similar but not identical half-pound samples: there were occasional results that fell just outside a ± 5 per cent. variation from the mean value for the pair, but the uniformity of the over-all average values showed that the variations were random, and, in fact, the inter-laboratory coefficient of variation for these results was approximately 5 per cent. The results may therefore be considered very satisfactory when viewed as a test of method.

The values obtained ranged between 13·0 i.u. per g and 33·2 i.u. per g for the individual mean vitamin-A potencies and between 3·7 i.u. per g and 14·4 i.u. per g for the individual mean carotene potencies. This range is typical of that known to exist between summer and winter butters. The widest range occurred in the Danish butters.

SUMMARY AND CONCLUSIONS

The chromatographic method of analysis for margarine of Boldingh and Drost,[5] as modified and subsequently adopted as the official method,[6] has been applied to a series

TABLE I

VITAMIN-A AND CAROTENE POTENCIES OF IMPORTED AND ENGLISH BUTTER

Composition			Vitamin-A potency		Carotene potency	
New Zealand butter, %	Australian butter, %	Danish butter, %	Unilever laboratory, i.u. per g	Government laboratory i.u. per g	Unilever laboratory, i.u. per g	Government laboratory, i.u. per g
100	—	—	22·9	24·0	9·9	9·3
100	—	—	23·7	24·7	9·8	9·2
100	—	—	23·6	—	9·9	—
100	—	—	20·0	—	9·4	—
100	—	—	21·9	23·9	9·7	9·4
100	—	—	22·8	22·2	10·5	10·1
100	—	—	33·3	33·1	15·2	14·8
100	—	—	33·4	32·0	12·8	13·9
100	—	—	24·5	22·9	10·1	10·0
100	—	—	26·8	28·1	13·3	13·8
100	—	—	21·8	23·0	13·2	14·4
100	—	—	21·5	21·9	13·2	13·4
100	—	—	23·4	22·0	12·9	12·5
100	—	—	24·1	23·0	12·3	12·4
100	—	—	21·7	21·2	14·4	12·0
100	—	—	22·0	19·9	13·4	12·5
100	—	—	23·8	22·8	14·3	14·5
100	—	—	24·7	22·7	13·6	12·7
—	100	—	23·5	24·7	12·6	11·9
—	100	—	21·9	20·9	11·3	10·5
—	—	100	29·4	31·7	10·8	8·8
—	—	100	30·2	30·7	8·0	8·3
—	—	100	28·6	30·2	9·2	9·1
—	—	100	30·7	31·8	8·7	9·0
—	—	100	30·0	—	9·2	—
—	—	100	27·8	30·0	9·4	9·6
—	—	100	29·6	—	10·2	—
—	—	100	26·5	—	9·9	—
—	—	100	30·9	31·6	9·8	9·6
—	—	100	17·5	16·4	4·6	5·0
—	—	100	15·8	13·9	4·3	4·6
—	—	100	15·6	14·3	4·4	5·5
—	—	100	16·1	15·0	4·0	4·5
—	—	100	14·9	12·2	4·3	4·5
—	—	100	18·7	18·5	5·7	5·6
—	—	100	15·4	13·3	4·3	4·1
—	—	100	13·5	13·3	3·7	4·3
—	—	100	13·7	13·8	3·5	4·0
—	—	100	14·6	11·5	3·9	3·8
80	—	20	27·9	32·9	11·2	11·6
80	—	20	22·0	24·2	9·2	9·2
80	—	20	22·1	20·4	11·6	12·4
80	—	20	22·5	20·3	11·6	10·5
80	—	20	26·3	23·0	12·3	11·2
80	—	20	22·2	19·8	12·5	12·1
80	20	—	22·0	21·0	12·5	12·4
—	80	20	20·5	18·7	8·6	8·5
—	80	20	24·7	23·5	9·5	9·8
—	80	20	23·4	22·6	10·1	10·6
—	80	20	26·8	25·8	12·1	11·2
60	—	40	32·7	29·5	12·7	12·4
60	—	40	27·2	26·3	9·5	9·5
60	—	40	26·6	26·7	11·9	11·0
60	—	40	20·5	18·5	9·7	8·5
60	—	40	17·1	16·3	9·8	9·6
60	—	40	19·4	18·5	9·6	9·4
60	—	40	20·4	18·2	7·8	7·8
60	—	40	18·9	18·3	9·7	8·7
60	—	40	17·5	19·4	8·9	8·4
60	—	40	18·7	18·9	8·4	8·2
60	—	40	18·8	19·3	9·0	8·6
60	—	40	20·5	21·0	11·4	11·4
60	40	—	24·3	24·1	12·4	12·7

Composition			Vitamin-A potency		Carotene potency	
New Zealand butter, %	Australian butter, %	Danish butter, %	Unilever laboratory, i.u. per g	Government laboratory, i.u. per g	Unilever laboratory, i.u. per g	Government laboratory, i.u. per g
60	40	—	25·3	25·3	12·2	9·4
—	60	40	22·2	19·3	9·8	9·3
—	60	40	21·1	20·6	10·2	10·0
—	60	40	16·4	15·2	4·1	4·1
—	60	40	22·2	21·6	8·2	7·8
—	60	40	22·7	21·1	7·7	7·5
—	60	40	20·1	20·7	8·4	7·9
—	60	40	20·3	19·7	9·4	8·5
—	60	40	17·1	18·7	8·0	8·3
40	60	—	30·2	28·4	12·9	13·5
40	60	—	29·4	28·2	12·8	13·0
40	60	—	31·2	30·2	12·8	13·4
40	60	—	24·9	25·6	12·9	13·6
40	60	—	23·5	23·2	10·7	10·9
40	—	60	25·8	27·3	9·7	8·5
40	—	60	25·2	27·3	8·7	8·1
40	—	60	25·4	28·8	9·6	8·1
40	—	60	25·7	28·7	9·4	9·0
40	—	60	25·7	27·5	9·5	8·7
40	—	60	29·1	31·3	9·7	9·4
40	—	60	25·6	26·3	9·9	9·6
40	—	60	24·5	—	10·0	—
40	—	60	26·8	—	9·0	—
40	—	60	28·9	28·7	10·3	9·9
40	—	60	28·0	28·2	9·1	8·7
40	—	60	29·3	29·8	8·4	8·8
40	—	60	27·5	27·1	9·9	9·1
40	—	60	28·0	26·5	9·2	8·8
40	—	60	27·2	25·6	10·2	9·2
40	—	60	26·6	26·1	11·0	9·6
40	—	60	26·9	26·0	10·0	9·7
40	—	60	25·4	24·5	10·2	9·8
40	—	60	26·5	26·5	10·4	10·2
40	—	60	27·5	29·3	10·1	9·7
40	—	60	30·1	26·4	10·7	9·8
40	—	60	27·8	26·5	10·1	9·8
40	—	60	27·0	26·0	9·6	9·8
40	—	60	32·4	29·8	11·4	10·6
40	—	60	26·4	26·1	9·0	8·5
40	—	60	23·6	23·6	8·8	9·0
40	—	60	29·1	27·0	11·5	11·2
40	—	60	16·9	17·7	7·3	7·2
40	—	60	17·2	18·5	7·3	7·2
English butters, Group 1			23·2	21·8	5·1	4·7
			18·7	17·7	4·2	4·0
			21·6	19·9	4·9	4·4
			20·7	19·7	4·7	4·2
English butters, Group 2			23·4	20·4	9·0	8·8
			22·4	20·9	9·3	9·0
			22·0	22·2	8·9	8·7
			23·1	22·2	9·4	9·0
			20·8	21·0	8·6	9·0
			20·9	20·9	9·0	9·1

of imported and English butters, and their vitamin-A and carotene potencies thereby determined.

This work was carried out collaboratively in two laboratories at the request of the Ministry of Food.

Agreement between the laboratories was good, and the mean vitamin-A and carotene potencies for the 116 butters examined were 23·6 i.u. per g and 9·5 i.u. per g, respectively.

We thank the Ministry of Food, the Government Chemist and the Directors of Unilever Ltd. for permission to publish these results.

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THE GOVERNMENT LABORATORY
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LONDON, E.C.2
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PORT SUNLIGHT

June 24th, 1955

Notes

THE DETERMINATION OF OLEIC ACID AND POTASSIUM OLEATE IN WHITE SPIRIT

METHODS for the direct titration of free and combined fatty acids in white-spirit solutions of alkali-metal based dry-cleaning soaps are described by Marshall.[1] Free fatty acid is determined first by titrating the sample with a 0·1 *N* solution of potassium hydroxide in a mixture of white spirit, *n*-butanol and ethanol, with phenolphthalein as indicator, and the total fatty acid is then determined by titration with a 0·1 *N* solution of hydrochloric acid in a white spirit - *n*-butanol mixture, with a special indicator. The recommended indicators for the second titration are mixtures of eosin with either xylene cyanol FF or malachite green, and the end-point is shown by a change in fluorescence in addition to a change in colour. A correction is applied to the titration with acid to allow for the difference in end-points between phenolphthalein and the special indicators. The fluorescent indicators have not proved to be entirely satisfactory in our hands and, further, continued exposure to the vapours of *n*-butanol during a long series of analyses proved to be unpleasant.

Palit[2] titrated soaps in mixtures of 1 : 2-glycols with higher alcohols and chlorinated solvents and noted that indicators did not always function in the order expected from their pH ranges in water. Similar effects occur in mixtures of white spirit and *iso*propanol, *e.g.*, thymol blue (pH range in water 1·2 to 2·8) turns yellow on the addition of acetic acid and red with a trace of mineral acid, whereas bromphenol blue (pH range 2·8 to 4·6 in water) shows a yellow colour with both acetic acid and mineral acid. Thymol blue is the most suitable indicator we have found for titrations with standard acid in white spirit - *iso*propanol mixtures. End-points are sharp and consistent provided that, at the end-point, the solution consists of a single phase and contains less than 5 per cent. of water by volume. In the presence of larger amounts of water, the colour change from yellow to red at the end-point is retarded and high titres are obtained.

The potassium chloride precipitated during the titration of soaps against hydrochloric acid in white spirit - *iso*propanol mixtures is rather flocculent and tends to mask the colour change of the indicator. Potassium perchlorate, which is precipitated more slowly and in a more crystalline form, does not have this effect, and so perchloric acid is used for the preparation of standard acid solutions as recommended by Palit.

METHOD

REAGENTS—

Standard 0·1 N *potassium hydroxide solution*—Dissolve 8 g of analytical-reagent grade potassium hydroxide in 20 ml of water and add 60 ml of ethylene glycol, 520 ml of *iso*propanol and 400 ml of white spirit. Complete solution is obtained on shaking and the loss of strength is about 2 per cent. in 4 weeks. In the absence of ethylene glycol the potassium hydroxide is difficult to dissolve, and at low water contents the solution is unstable owing to gradual precipitation of the alkali.

The potassium hydroxide solution is standardised as follows. Weigh out accurately 0·3 to 0·7 g of analytical-reagent grade benzoic acid that has been dried for 2 hours at 100° C. Moisten with 5 ml of a (1 + 1) mixture of ethylene glycol and *iso*propanol previously neutralised to phenolphthalein. The benzoic acid partly dissolves at this stage, the remainder dissolving as titration proceeds. Titrate with the potassium hydroxide solution, using 3 drops of 0·5 per cent. phenolphthalein in 70 per cent. *iso*propanol or ethanol as indicator. Two phases are present at the beginning of the titration, but these become mutually soluble as the end-point is approached. If

necessary, sufficient neutral *iso*propanol should be added to ensure a single phase solution at the end-point.

$$\text{Normality of potassium hydroxide} = \frac{\text{weight of benzoic acid} \times 1000}{\text{titre} \times 122{\cdot}05}.$$

Consistent results are obtained in the standardisation, as the following example shows—

Weight of benzoic acid, g	0·3318	0·6860	0·5770
Titre, ml	23·50	48·65	40·90
Normality	0·1158	0·1156	0·1156

Standard 0·1 N *perchloric acid solution*—Dissolve 16 g of 60 per cent. perchloric acid in a mixture of 600 ml of *iso*propanol and 400 ml of white spirit. Titrate measured volumes of the standard alkali solution with the solution of perchloric acid, using 3 drops of 0·1 per cent. thymol blue in *iso*propanol or ethanol as indicator and titrating to the red end-point. Replicate titrations are within the range ± 0·1 ml from the average. The solution loses approximately 2 per cent. of its strength in 4 weeks, presumably owing to the formation of esters.

PROCEDURE—

Suitable aliquots of dry-cleaning soaps in white spirit are taken by pipette and mixed with about twice their volume of *iso*propanol previously neutralised to phenolphthalein. One sample is titrated with the standard potassium hydroxide solution to the phenolphthalein end-point and the other is titrated with the standard perchloric acid solution to the red end-point of thymol blue.

Both titrations may be carried out on the same sample if desired, there being no need to apply a correction. This is shown by the following examples, in which weighed portions of oleic acid were dissolved in white spirit and titrated first with standard alkali and then with standard acid—

Weight of oleic acid, g	1·3370	0·7062	0·9324
Volume of 0·1 *N* potassium hydroxide, ml ..	48·07	25·25	33·45
Volume of 0·1 *N* perchloric acid, ml	48·00	25·30	33·35

RESULTS

The neutralisation equivalent of a sample of oleic acid was determined by the usual method[3] and found to be 282. Weighed amounts of the oleic acid were dissolved in white spirit and titrated by the method given above. The calculated equivalent weight of the oleic acid in separate determinations was: 278·3; 279·8; 278·9; 278·3; 278·0; 277·2; average 278(·4).

Because of the low equivalent weight shown in these titrations, determinations of free fatty acids in dry-cleaning soaps tend to be high. A similar discrepancy is shown by Marshall's figures.[1] When known materials are used, the oleic acid equivalent of the standard alkali should always be determined to eliminate this error.

A sample of the same oleic acid was converted to its potassium salt, and analysis by the usual methods showed a total alkali content of 14·9 per cent. (as K_2O) of which 0·1 per cent. was free carbonate, there being no free caustic alkali. The corresponding equivalent weight of the soap was 316. Weighed amounts of the dry soap were wetted with a few drops of water and dissolved in *iso*propanol. Aliquot portions were added to white spirit and the equivalent weight was determined by titration against the perchloric acid with thymol blue as indicator. The following values were obtained: 314·4; 319·2; 316·2; 317·7; 317·8; average 317(·1).

The methods described have been applied in laboratory work to dry-cleaning soaps based on mixtures of oleic acid with potassium oleate peptised by small amounts of water, but are no doubt applicable to other fatty acids and alkali-metal soaps. The analysis is not affected by the presence of auxiliary solvents, such as alcohols, Cellosolves and Carbitols, in the dry-cleaning soap.

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2. Palit, S. R., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 246.
3. Hurst, G. H., and Simmons, W. H., "Textile Soaps and Oils," Third Edition, Scott Greenwood & Son Ltd., London, 1921, p. 159 *et seq.*

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September 6th, 1955

THE DETERMINATION OF MILK SOLIDS-NOT-FAT IN ICE-CREAM

A LEGAL standard for ice-cream led to considerable difficulty in the determination of the milk solids-not-fat (milk s.n.f.) and in a laboratory handling large numbers of samples, a method had to be developed that was both accurate and rapid.

Milk solids-not-fat can be determined in ice-cream by a determination of lactose, protein, ash and possibly calcium and phosphate in the ash. These determinations are time-consuming and liable to errors, particularly the lactose, as in the early days of the standard being enforced, lactose as such was a common ingredient, and is still in use to-day, although to a much lesser degree. Also, the presence of glucose or invert sugars complicates the determination.

Trials were made on laboratory prepared ice-cream mixes of known composition, use being made of the well known formol titration of milk proteins by Richmond's classical method, and the results were so satisfactory that the method has been adopted and has been in constant use over the past 3 years.

METHOD

Weigh 10 g of the ice-cream sample accurately into a porcelain dish. Add 1 ml of phenolphthalein solution and titrate with 0·1 *N* sodium hydroxide, the end-point being indicated by a faint pink colour.

To this neutralised ice-cream add 3 ml of 40 per cent. formaldehyde solution, stir the mixture and then titrate again to neutrality.

Separately titrate 3 ml of the formaldehyde solution with the 0·1 *N* sodium hydroxide to determine the blank, and deduct the titre value from that for the sample.

CALCULATION—

The factor to convert the amino-acid value or its equivalent, the aldehyde figure, into milk proteins has been determined by a number of workers, but the factor given by Richmond[1] (0·17) is still widely used in the dairy trade and so it was adopted.

If x = ml of 0·1 *N* sodium hydroxide required for neutralising after addition of formaldehyde, and

y = ml of 0·1 *N* sodium hydroxide required to neutralise 3 ml of formaldehyde solution,

then $(x - y) + 10$ = aldehyde figure,

and $(x - y) \times 10 \times 0{\cdot}17$ = percentage of milk protein.

Skimmed-milk powder, *e.g.*, milk solids-not-fat, contains an average of 30 per cent. of milk protein.

Then—

$$\frac{(x - y) \times 1{\cdot}7 \times 100}{30} = (x - y) \times 5{\cdot}67 = \text{percentage of milk s.n.f.}$$

This factor, $(x - y) \times 5{\cdot}67$, was used throughout the experimental tests described below.

EXPERIMENTAL

Some 1-g portions of dried skimmed-milk powder were dissolved in water, and the recoveries were 0·997, 0·991, 0·997, 1·002, 0·998 and 1·000 g.

To test the effect of cereal flours, 1-g portions of dried skimmed-milk powder were mixed with 0·03 g of wheat flour (approximately 3 per cent. of flour is commonly used in ice-cream), and the recoveries were 0·998, 0·997, 1·002, 1·000, 0·996 and 0·998 g.

TABLE I

RESULTS ON ICE-CREAM MIXES

	Milk s.n.f. in mixture *a*		Milk s.n.f. in mixture *b*		Milk s.n.f. in mixture *c*		Milk s.n.f. in mixture *d*	
	operator A, %	operator B, %	operator A, %	operator B, %	operator A, %	operator B, %	operator A, %	operator B, %
	7·40	7·48	9·91	10·00	5·00	5·00	Nil	Nil
	7·46	7·46	10·01	9·95	4·98	5·00		
Average	7·44	7·47	9·96	9·97	4·99	5·00		

To test the effect of gelatin, 1-g portions of dried skimmed-milk powder were mixed with 0·005 g of powdered gelatin (approximately 0·5 per cent. of gelatin is used in ice-cream), and the recoveries were 1·000, 1·001, 0·998, 0·996, 1·000 and 1·002 g.

Ice-cream mixes containing (*a*) 7½ per cent., (*b*) 10 per cent., (*c*) 5 per cent., and (*d*) nil milk solids-not-fat were prepared in the laboratory; the mixes contained the usual fat, sugar, filler, emulsifier and stabiliser. Samples of these mixes were submitted to two individual operators, (A) and (B), who were not aware of their composition. The results obtained are shown in Table I.

These results are in close agreement with the known compositions, and no interference is shown by other protein materials commonly used.

After extensive use with hundreds of samples, this method has been found to give very satisfactory results, with the factor 5·67 to multiply the formol titration value.

In addition, one other useful point is that the protein can be rapidly calculated if needed for the factor used to allow for the volume of precipitate in clarification of solutions for sugar determinations.

I thank Mr. L. V. S. West for his assistance in carrying out many of these tests.

REFERENCE

1. Richmond, H. D., "Dairy Chemistry," Third Edition, Charles Griffin & Co. Ltd., London, 1920, p. 179.

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May 27th, 1955

THE REDUCTION OF NITRO GROUPS IN ORGANIC COMPOUNDS BEFORE DIGESTION BY KJELDAHL'S METHOD

SEVERAL methods have been proposed for the reduction of nitro groups in order to extend the scope of Kjeldahl's method, the most widely used being that of Friedrich,[1] in which red phosphorus and hydriodic acid are used. This method is lengthy, for after reduction it is necessary to boil until all the free iodine is expelled before continuing the normal digestion. It would be more convenient to use some reagent effecting immediate reduction and then permitting the normal digestion to be continued with less delay. We have examined two reagents that seemed suitable for this purpose, titanous and chromous salts. As might be expected, the chromous salt (chloride or sulphate) proved to be the more efficient, and attention was confined to this reagent.

Two compounds containing nitro groups were examined and satisfactory results were obtained (see Table I). The reduction of azobenzene was also attempted, but the reaction does not appear to go to completion. The reagent might possibly be applied to the reduction of other groups.

TABLE I

DETERMINATION OF NITROGEN

	Nitrogen expected, %	No. of tests	Average nitrogen found, %
m-Dinitrobenzene ..	16·67	9	16·59
p-Nitroaniline	20·29	3	20·33
Azobenzene	15·38	7	14·92

Beet[2] has shown recently that oxidation with potassium permanganate can be used successfully for accelerating Kjeldahl digestions and has the advantage that the end of the reaction is indicated by the permanganate colour remaining unaffected. It was thought that dichromate might behave likewise and that it would then be possible to utilise the chromic salt already in the solution by oxidising it to dichromate with additions of potassium persulphate. A more stable end-point would be expected than that given by permanganate. However, it was found that, when potassium dichromate was added in the same way as permanganate in Beet's method, low results were obtained even with simple compounds unless a very prolonged digestion time was used; this procedure was not examined further.

METHOD

REAGENT—

Chromous chloride solution—Dissolve 15 g of chromic chloride hexahydrate in 100 ml of 2·5 *N* sulphuric acid. Transfer to a 250-ml bottle and add mercury containing about 2 per cent. of zinc. Add light petroleum to a depth of about 5 cm. Shake the bottle occasionally over a period of 30 minutes; a blue solution should be obtained.

PROCEDURE—

Dissolve 4 to 5 mg of the nitro compound in 1 ml of sulphuric acid in a Kjeldahl flask, add a small piece of solid carbon dioxide to expel the air from the flask and run in 2·0 to 2·5 ml of chromous chloride solution, swirling the contents of the flask continuously. Shake the flask and remove the excess of water by boiling. Add 0·5 g of potassium sulphate and 0·075 g of mercuric sulphate. Digest and steam-distil in the usual way.

A blank should be carried out in exactly the same manner, 5 mg of sucrose being used.

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DEPARTMENT OF CHEMISTRY
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November 23rd, 1955

Book Review

THE QUANTITATIVE ANALYSIS OF DRUGS. By D. C. GARRATT, B.Sc., Ph.D., F.R.I.C. Second Edition. Pp. xvi + 670. London: Chapman & Hall Ltd. 1955. Price 70s.

The first edition of this book was published under the title "Drugs and Galenicals: their Quantitative Analysis" and was reviewed in *The Analyst* (1938, **63**, 74).

The title has been changed because of the restricted meaning of the term "galenicals" and their diminished importance in medicine.

The book is essentially a collection of chemical and physical methods for the analysis of drugs; microbiological methods are not included. The methods have been well chosen and the descriptions are not merely lists of directions but are accompanied by numerous hints and suggestions for overcoming difficulties; these are doubtless culled from the laboratory notebooks accumulated by Dr. Garratt in his many years of experience in this field. For these reasons the book will be indispensable to practising analysts concerned with drug analysis.

Dr. Garratt has clearly carried out most successfully the task that he set himself, that is, to write a book on quantitative methods of analysis, but one wonders why he has chosen to limit the book in this way, since the absence of qualitative tests for identification and purity seems to detract from its value to some extent; indeed the restriction seems somewhat artificial. There are many quantitative methods that are incomplete without additional qualitative tests for purity, *e.g.*, the extraction and weighing of a base after making a solution of its hydrochloride alkaline has little value unless the base is identified and its purity determined. Some of the methods in this book and in official publications are open to the criticism that their use does not lead to the detection of closely related compounds that may occur, for example, in synthetic drugs. Such compounds may react quantitatively in a manner very like that of the drug itself.

For the detection and determination of such impurities chromatography would appear to be the most suitable and indeed often the only method, yet, in spite of its rapidly extending use in other directions, a search of this book provided only three examples of its use. It is evident that the method has not found much application in the pharmaceutical field, possibly because it does not lend itself easily to precise description in official publications.

While one can be whole-hearted in recommending the book to the practising analyst, one cannot feel quite so enthusiastic about advocating its use by the student. There is little detailed description of general physical or chemical methods. The final chapter includes, almost as an afterthought, sections on certain physical methods. Light-absorption methods, electrometric titrations, emission spectrography and polarography (but *not* chromatography) occupy only

nine pages; the treatment is therefore necessarily somewhat cursory and no illustrations or diagrams of apparatus are included.

The arrangement of the material is largely in the alphabetical order of the names of the drugs as in the B.P., pharmaceutical preparations being included under the parent drug. While this arrangement has the advantage of simplicity, it debars the author from any general treatment of related groups, such as the natural drugs containing alkaloids or glycosides. Nevertheless, the author has wisely made certain exceptions to this arrangement and has collected, for instance, oils, fats and waxes, and essential oils into separate chapters.

Methods for the determination of water, traces of metallic impurities, alkaloids and alcohol, titrations in non-aqueous solvents, the extraction of organic chemicals from viscera and micro-analytical tests for the presence of extraneous matter (the "filth test") in foods and drugs are all to be found in appendixes. A supplement gives revisions of the text made necessary by the publication of the B.P.C. 1954.

The difficulties of a logical arrangement of material in a book of this kind are great, but an adequate index will, to some extent, compensate for them. For this reason the index might, with advantage, be expanded.

The book is clearly and well written and produced; misprints are remarkably few. Such criticisms as have been made in this review are of minor importance compared to the undoubted excellence of the book as an aid to the drug analyst. NORMAN EVERS

Publications Received

SYNTHETIC ION-EXCHANGERS: RECENT DEVELOPMENTS IN THEORY AND APPLICATION. By G. H. OSBORN, F.R.I.C. Pp. x + 419. London: Chapman & Hall Ltd. 1955. Price 30s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, 1955–1956. Pp. vi + 296. London: The Pharmaceutical Press. 1955. Price 15s.

BIOCHEMICAL PREPARATIONS. Volume 4. Editor-in-Chief: W. W. WESTERFELD. Pp. viii + 108. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1955. Price $3.75; 30s.

PLANT PHYSIOLOGY. By MEIRION THOMAS, M.A., F.R.S., F.R.S.E., S. L. RANSON, B.Sc., and J. A. RICHARDSON, M.Sc., A.Inst.P. Fourth Edition. Pp. xii + 692. London: J. & A. Churchill Ltd. 1956. Price 45s.

SPEKTROCHEMISCHE BETRIEBSANALYSE. By Dr. HEINRICH MORITZ. Second Edition. Pp. xvi + 240. Stuttgart: Ferdinand Enke Verlag. 1956. Price (paper) DM 52; (cloth boards) DM 55.

MECHANISM OF ORGANIC CHEMICAL REACTIONS. By E. DE BARRY BARNETT, D.Sc., F.R.I.C. Pp. xii + 289. London and Glasgow: Blackie & Son Ltd. 1956. Price 30s.

QUALITATIVE AND VOLUMETRIC ANALYSIS. By J. C. GIBLIN, B.Sc., A.R.I.C. Second Edition. Pp. xiv + 183. London, New York and Toronto: Longmans, Green & Co. Ltd. 1955. Price 8s.

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The title should be brief but descriptive.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Descriptions of new methods should be supported by experimental results showing their precision and selectivity.

Generally, the best order of presentation is as indicated below—

(*a*) Synopsis.
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(*e*) Presentation of results.
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Tables, diagrams, etc.—The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns may often be arranged horizontally. No lines should be ruled in tables in the manuscript. Tables should be supplied with titles and be so set out as to be understandable without reference to the text.

Tables or graphs may be used, but not both for the same set of results, unless important additional information is given by so doing.

In general, graphs should have a reasonable number of co-ordinate lines, and not only the two main axes. Graphs consisting of straight lines passing through the origin, such as calibration curves, should not be submitted: instead, an equation should be given in the text.

Diagrams and graphs should be drawn in Indian ink on Bristol board, stout paper or tracing cloth, not larger than foolscap size. The use of squared paper should be avoided if possible, as it often leads to poor reproduction. Red, orange or brown ruled paper must not be used; if it is necessary to use ruled paper, blue or green rulings may be used, as these colours do not reproduce in block-making. All lettering should be inserted lightly in black lead pencil at the appropriate place on the diagram, and will be replaced by type in block-making. All lines in Indian ink should be firmly drawn and sufficiently thick to stand reduction. If the artist is uncertain in his free-hand work on graphs, he should submit a careful pencil drawing that can be traced.

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Percentage concentrations of solutions should be stated as "per cent. w/w" (alternatively "g per 100 g"), as "per cent. w/v" (alternatively "g per 100 ml") or as "per cent. v/v." Concentrations of solutions of the common acids, however, are often conveniently given as dilutions of the concentrated acids; they should then be given in the form "diluted hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might be equivalent to *either* 1 + 4 *or* 1 + 3.

References—References should be numbered serially in the text by means of superscript figures, *e.g.*, Dunn and Bloxam[1] or Mitchell,[2] and collected in numerical order under "REFERENCES" at the end of the paper. They should be given, with the authors' initials, in the following form—

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933, 52,** 189T.
2. Mitchell, C. A., *Editor*, "Allen's Commercial Organic Analysis," Fifth Edition, J. & A. Churchill Ltd., London, **1932**, Volume 9, p. 149.

For books, the edition (if not the first), the publisher, and place and date of publication should be given, followed by volume or page number, or both if required.

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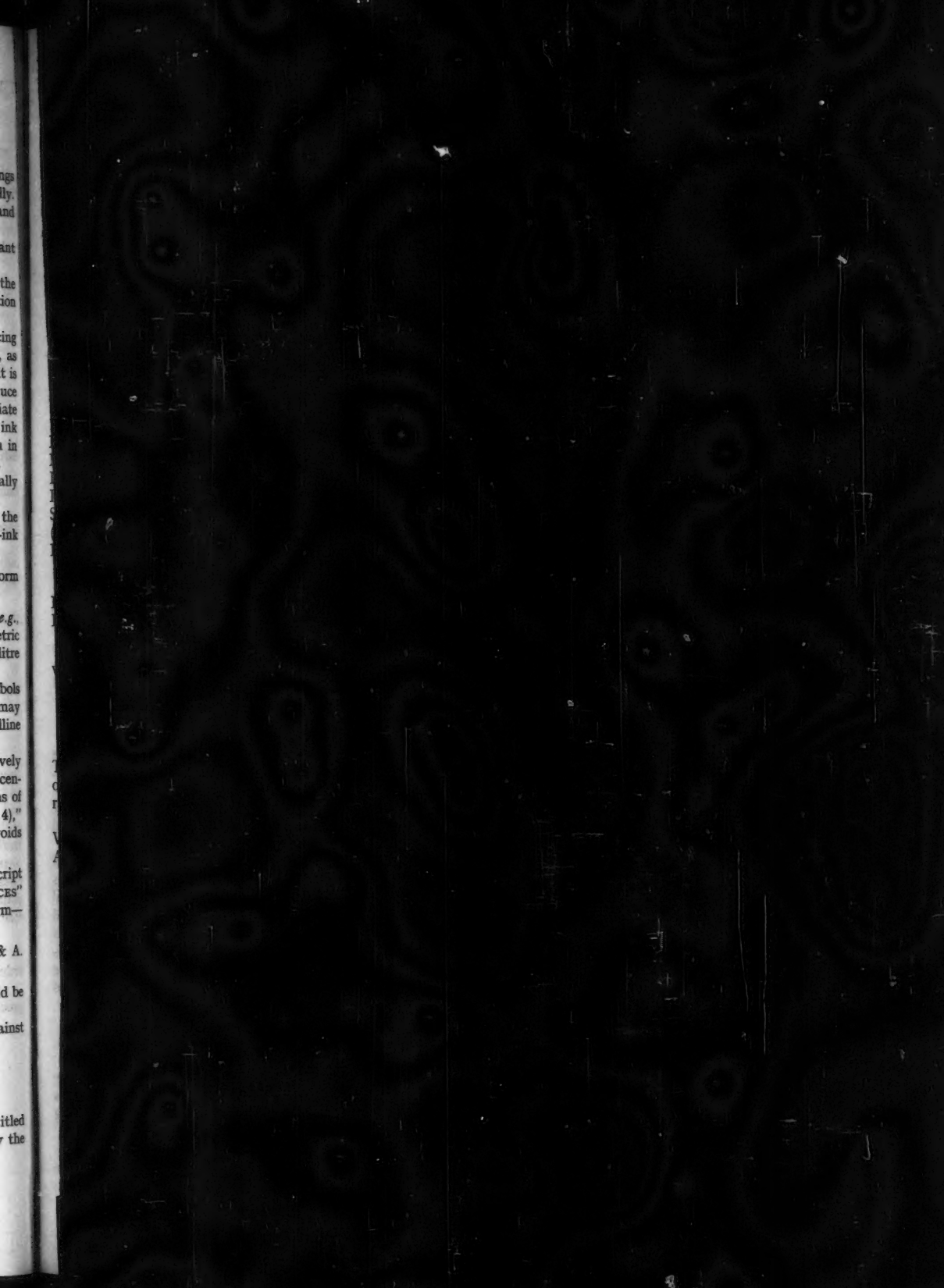
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